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Introduction

There are not many diseases which have attracted the attention of such a large range of specialists within the scientific community as has malaria. Apart from the traditional medical fields (microbiology, epidemiology, medicine, pathology and pharmacology) those employed in biological sciences, civil engineering, economists, mathematicians, sociologists, health educators, geographers, ethnologists, etc. have all made some contribution to our present knowledge of the disease. Yet, in spite of the tremendous amount of observations made, experimentally and in the field, there is still much to be learned about the parasite, the vector, the reaction of the host and the dynamics of transmission. It is true that since DDT was introduced as an effective tool for the control of malaria, many epidemiological observations made over the past forty years or more have been forgotten and will have to be looked at again. However, one should not forget that the environment, and society itself, have undergone considerable change and, in many instances, new ecological conditions have developed. Mankind's powerful technology is permanently interfering with the natural processes thereby perturbing the environmental equilibrium. In the re-establishment of this equilibrium there has been some genetic selection among the affected vertebrate, and more particularly invertebrate species, resulting in the development of a population of some species capable of surviving the new conditions created.

At the time countries were urged to initiate public health programmes aimed at eradicating malaria, it seemed that, with a tool as powerful as DDT, malaria transmission could be interrupted and, in the absence of new infections, the parasite reservoir depleted, mostly by natural death of the plasmodium parasitizing man and partly by antimalaria drugs. An exception was made for *Plasmodium malariae*, as this species seldom causes serious problems of an epidemiological nature. Africa, south of the Sahara, was not included in the global malaria eradication programme due to the holoendemic type of malaria in many places of that part of the continent and to the lack of infrastructure.

An analysis of experience gained in the past 25 years in programmes aimed at eradicating the disease indicates that, in addition to the technical, operational and administrative problems usually cited as reasons for non-interruption of transmission or slow progress, there were many situations in which the methods applied simply did not work.

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1 Prepared by Dr T. Lepes, Director, Malaria Action Programme, World Health Organization, Geneva for the PAHO/RACMR, Costa Rica, June 1980

MAP.16.4.80.
It is true that, in most instances of non-response of malaria to control measures there was a mixture of technical problems and operational and administrative shortcomings. It should be recognized that the technical services were very quick to blame resistance of vectors to insecticides or parasites to drugs as the main reason for non-interruption of transmission, although sometimes the operational and administrative difficulties were underlined. It is interesting to note that the intensity of transmission, or rather the intensity of man-mosquito-contact and its seasonality, has seldom been considered. As for the operational aspects, more emphasis has been placed on insecticide coverage than on the timing and frequency of spraying. For surveillance, efforts have been directed more towards the number of slides collected in terms of quantity rather than on the quality of surveillance in terms of epidemiological indications.

While it is not the purpose of this paper to analyse in detail all the shortcomings of malaria eradication programmes, we must learn the lessons which our experience of the past 25 years can teach us, and keep before us the question - why did the malaria eradication programmes not progress in accordance with our expectations?

1. Present status of malaria control in the world

Difficulties encountered by many of the countries that had initiated malaria eradication programmes in the mid and late fifties made it imperative to review a certain number of these programmes in greater detail. This ultimately led to the development of a revised strategy for malaria eradication (adopted by the Twenty-second World Health Assembly in 1969). The revised strategy included two essentially new elements: (1) the necessity of making a detailed review of each programme which would be revised in accordance with the actual epidemiological situation and (2) the replanning of antimalaria activities would be based on financial and manpower resources available to the countries concerned. This meant, in fact, that the time-limited nature of malaria eradication programmes, which had been insisted on initially, had to be ignored. At the same time, due to the shortage of financial and manpower resources in most developing countries and the fact that many other health priorities had to be met, it was simply not possible to utilise the epidemiological approach to comply with the principle of total coverage. Under these circumstances it was clear that some countries had to convert their malaria eradication programmes into malaria control programmes. Some countries did this both in title and substance but many retained the title of eradication and did not make substantial changes in the antimalaria activities. Consequently malaria resurgence occurred during the period 1972-1976 and, in some areas, it took epidemic form.

Since the mid-seventies much has been written in the news media and in health literature and many discussions have taken place at various meetings on malaria. Malariologists were blamed for their over-optimistic and selfish drive towards eradication. The programme was described as a "failure" in contrast to the successful smallpox eradication campaign. I do not think that malaria eradication was a failure, nor is malaria comparable in any aspect to smallpox. Nevertheless, the deteriorating situation required urgent action on the part of governments of malarious countries and by the World Health Organization.
Considering the growing technical problems i.e. resistance of vectors to insecticides and *P. falciparum* resistance to antimalaria drugs, the ever-persisting operational and administrative difficulties manifested in the lack of trained and experienced national personnel, financial difficulties, inaccessibility of certain malarious areas and the fact that in countries of Africa south of the Sahara not much could be done with the tools available at present, the Director-General submitted to the Thirty-first World Health Assembly a malaria control strategy which could reasonably be accepted by all malaria-affected countries. For obvious reasons it was mainly a defensive strategy but it is flexible enough to allow all levels of control from prevention of mortality to eradication of the disease. The strategy sets out four conditions which must be fulfilled if any type of malaria control is to be successful: (1) the expression of national will demonstrated by a political decision to control the disease; (2) national decision to provide long-term support to malaria control; (3) that malaria control should be an integral part of country health programmes and (4) that community participation, including multisectoral cooperation, must be secured.

In the technical implementation of malaria control/eradication programmes flexibility and the epidemiological approach have been stressed as two basic principles to be followed. In view of the spreading of malaria resurgence in various parts of the world, it was emphasized that the following types of activities would have to be undertaken practically simultaneously: (1) control of epidemics and prevention of further spreading of resurgence; (2) development of long-term malaria control plans; (3) training and (4) research.

It is fully realized that, strictly speaking, concepts, guiding principles and general declarations do not, in themselves, control any kind of disease, least of all malaria. Nevertheless, these are important pre-requisites for any concerted action against a widespread public health problem such as malaria.

Before touching on the question of the technically sound reorientation of malaria control/eradication programmes, let us review briefly the status of endemicity and the progress of control in the past few years.

For reasons of convenience all areas and countries of the world could be described as: those with no risk of malaria transmission; those with minimal risk; those with moderate and high risk of transmission. In Table I it can be seen that, excluding China, 1,618,380,000 people live in areas with moderate to high risk, 110,790,000 in areas of minimal risk and 400,760,000 live in malaria-free areas. This means that, of the two-thirds of the world’s population originally exposed to the risk of malaria infection, roughly 75% is still under moderate to high risk.

When it comes to the number of cases reported by malarious countries the situation is somewhat better, although the figures presented in Table II should be considered with caution, as they only represent a general trend and are by no means a true reflection of the persisting parasite reservoir. Figures for Africa south of the Sahara, although available, have not been included in Table II for the simple reason that these figures do not cover the total rural population nor do they represent microscopically confirmed cases of malaria.
TABLE I. SUMMARY OF STATUS OF MALARIA SITUATION ACCORDING TO LEVEL OF MALARIA RISK BY REGION
(As at mid-year 1978) - Population figures in millions

<table>
<thead>
<tr>
<th>Region</th>
<th>Total no. of countries or areas</th>
<th>Estimated population 2</th>
<th>Countries or areas where malaria was endemic 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. of countries or areas</td>
<td>Population originally at risk</td>
<td>Risk nil countries or areas</td>
</tr>
<tr>
<td>Africa</td>
<td>47</td>
<td>336,18</td>
<td>43</td>
</tr>
<tr>
<td>Americas</td>
<td>49</td>
<td>586,50</td>
<td>34</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>10</td>
<td>992,02</td>
<td>8</td>
</tr>
<tr>
<td>Europe</td>
<td>38</td>
<td>822,57</td>
<td>17</td>
</tr>
<tr>
<td>Eastern Mediterraean</td>
<td>24</td>
<td>249,20</td>
<td>23</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>38</td>
<td>303,57</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>3290,04</td>
<td>143</td>
</tr>
</tbody>
</table>

1 Taking 1947 as reference year
2 Based on UN Monthly Bulletin of Statistics, VOL. XXXIII, No. 7, 1979
3 Excluding China
TABLE II. NUMBER OF MALARIA CASES$^1$ (IN THOUSANDS) REPORTED DURING THE PERIOD 1972-78 BY REGION

<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Americas$^3$</td>
<td>285</td>
<td>280</td>
<td>269</td>
<td>357</td>
<td>379</td>
<td>399</td>
<td>465</td>
<td></td>
</tr>
<tr>
<td>Europe$^3$</td>
<td>13</td>
<td>9</td>
<td>7</td>
<td>13</td>
<td>41</td>
<td>119</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>South-East Asia$^3$</td>
<td>1816</td>
<td>2686</td>
<td>4162</td>
<td>6096</td>
<td>7296</td>
<td>5552</td>
<td>4264</td>
<td></td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>830</td>
<td>746</td>
<td>480</td>
<td>424</td>
<td>347</td>
<td>227</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>Western Pacific</td>
<td>171$^2$</td>
<td>201$^2$</td>
<td>179$^2$</td>
<td>188$^2$</td>
<td>211$^2$</td>
<td>4464</td>
<td>3422</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3115</td>
<td>3922</td>
<td>5097</td>
<td>7041</td>
<td>8274</td>
<td>10761</td>
<td>8370</td>
<td></td>
</tr>
</tbody>
</table>

$^1$The information provided does not cover the total population at risk in some instances.

$^2$Excluding China

$^3$Microscopically confirmed cases
From the figures quoted in the Tables overleaf, it is evident that in countries of the Americas the number of cases almost doubled during the period 1972-1978. However, the great majority of reported cases come from eight countries (Bolivia, Colombia, El Salvador, Guatemala, Haiti, Honduras, Nicaragua and Peru). The sudden increase in Europe since 1976 was caused by the malaria outbreak in Turkey. The most serious resurgence of malaria occurred in countries of South-East Asia (Burma, India, Sri Lanka) but since 1976 a considerable reduction in the number of cases has been reported in India and Sri Lanka. Malaria in countries of the Eastern Mediterranean Region shows a downward trend, although surveillance activities have not been carried out regularly in some countries and therefore this trend may be misleading. Some overall increase has also been reported from countries of the Western Pacific Region, in which the malaria cases reported from China were included for the first time (4 200 000 cases in 1977 and 3 096 000 in 1978).

Generally it should be stated that the global epidemiological situation has not changed significantly and continues to be a matter of serious concern.

In addition to the general trend observed globally, data available for individual countries indicate that, even in countries where progress is being made, there are areas in which malaria transmission is "resisting" the anti-malaria measures applied. It would not be untrue to state that, for many areas, unless the methods of control and their application are changed or modified there will be no substantial progress towards eradication of malaria.

2. Research strategy

Before going into details of the research strategy to be pursued, there are certain issues or principles to be considered.

In carrying out malaria eradication programmes, the responsible national services have usually followed the "instructions" or guidelines developed by the Expert Committee on Malaria which were: total coverage spraying by insecticides twice or three times yearly and the collection and examination of blood slides of all fever cases and cases with a history of fever and the provision of antimalaria drugs for such cases. No doubt this simple technique worked well in some instances but in many others it failed. It is quite correct to maintain "eradication" as the ultimate goal of malaria control programmes but control methods must be adjusted to local ecological and other conditions necessitated by the resources available and the existing infrastructure. To select the most appropriate method of control for most areas it is necessary to have additional epidemiological field research. Such field research requires, in turn, personnel devoted to this task. It could be postulated, therefore, that all national malaria services should create posts within the service for field research activities. Depending on the size of the programme and the problems encountered, posts should be secured for one or more professional staff and for the technicians necessary.
There is no doubt that scientific communities in all countries would be very ready to cooperate with the "operational services". So far this kind of cooperation has been the exception rather than the rule. However, it must be admitted that without the contribution of scientific research malaria control may drag on for a long time. It is imperative, therefore, to involve the scientific community to the extent possible, first at national and then at regional and global level. I could continue to speak about details important for malaria control and for public health as a whole for creating a permanent liaison between operational services and the scientific community, but I am sure I am preaching to the converted.

It is known that the principal objectives of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases are the development of new tools for chemotherapy and immunization or improvement of existing ones, the development of diagnostic tests and procedures, the strengthening of research capabilities and training activities. Malaria is one of the six diseases included in the Programme with the proportionately highest budgetary allocation. There are three Scientific Working Groups: Chemotherapy, Immunology and Applied Field Research (CHEMAL, IMMAL and FIELDMAL). Each one has a detailed strategic plan of research. The FIELDMAL plan is annexed to this paper - others can be obtained on request.

Laboratory-based research certainly requires continuous stimulation and support but, in view of experience in the development of new antimalaria drugs and of an immunizing agent applicable to human malaria, national services should be aware that neither of these new tools will be available in the near future. The situation is similar as regards the development of new chemicals, either for house spraying, larviciding or space application and for new biological agents or methods of genetic control. It is important, therefore, to rely on existing tools and methods for the control of malaria and select them as appropriate to each situation.

The strategic plan for applied field research in malaria includes a lengthy list of possible topics for research. Although it is rather general in nature it is a useful guide to the national services in reviewing the impact of antimalaria operations carried out in different areas. Furthermore, this plan sets out the steps to be followed in evaluating each suspected problem and the approaches to be used in attempting to clarify them and to find possible solutions.

As far as the Americas are concerned, there is a long-standing tradition in malaria research which includes applied field research. The existing national institutions and expertise are capable of undertaking, through cooperative efforts, any type of research. In fact, at the moment much is being done on control methodology, entomology, serology and chemotherapy.

The problems encountered in countries of the Americas could be divided generally into three main categories:

1. Insecticide resistance and evasive behaviour of vectors;
2. Resistance of P. falciparum to chloroquine;
3. Operational problems linked with control methods applied, their frequency and timing of application. Closely linked with the above problems are accessibility of the terrain, community participation and timely release of funds for purchase of necessary supplies and equipment. This latter aspect, being an administrative problem, is closely related to operations requiring special attention.

3. Insecticide resistance

The physiological resistance of anopheles has undoubtedly been the principal technical problem and one of the main obstacles to the interruption of transmission encountered in malaria control/eradication programmes. Out of more than 80 anopheline species known to be vectors of malaria, 53 species have developed resistance to one or more insecticides. In fact, 37 species have developed resistance to DDT, 46 to DDT/dieldrin, 13 to organophosphorous compounds and five to carbamate insecticides. In view of the continuous and extensive application of insecticides in agriculture, which is particularly intensive in areas such as Central America, the situation can only worsen.

Considerable research has been done in the past few years to try to clarify the mechanisms involved in the physiological resistance of anopheles. It is now known, for example, that in A. albimanus, the resistance to organophosphorous and carbamate insecticides is caused by insensitivity of acetyl cholinesterase. Increased metabolism is another example by which the increased synthesis of DDT dehydrochlorinase rapidly converts the toxic molecule of DDT into DDE, non-toxic to mosquitoes. However, it should be pointed out that, while the mechanisms of resistance of some anopheline species has been well investigated, due to difficulties encountered in the rearing of some species in the laboratory, we do not as yet have the full picture of resistance in all malarious areas of the world. It is, of course, a complex question and one wonders how much priority should be given to this kind of research, bearing in mind the scarcity of institutions and research workers in this field.

The testing of vectors' susceptibility has been carried out for more than 20 years, initially using the Busvine-Nash method and, later on, the standard WHO test kits with pre-prepared impregnated filter papers. For routine monitoring, the use of "critical concentrations for a given period" has been introduced. For some vector species, such as A. maculipennis complex, critical concentration may require different exposure times. With organophosphorous compounds "critical concentration" may not be sufficiently determinant of the susceptibility-resistance status of the vector. Therefore repeated tests with different concentrations and at different periods of exposure should be applied.

Too much emphasis has been placed on susceptibility testing and not enough on the epidemiological importance of vector resistance. To cite one example, there was the resistance of A. stephensi in south Iraq (Basrah city) in 1963 where, in the absence of any other insecticide, DDT was applied in spite of resistance of the vector to that insecticide. However, a detailed epidemiological analysis of malaria cases following the residual house-spraying showed that, 15 days after the spraying was carried out, malaria declined very considerably and, in many areas of the city, transmission was even interrupted.
As already mentioned, no new "miracle" insecticide is expected to be developed in the near future. One should also not expect significant changes in the use of insecticides in agriculture in the foreseeable future. This means that we shall have to use existing insecticides for malaria control/eradication. However, there is still place for more appropriate use of the insecticides available in accordance with the results obtained from additional epidemiological research which should facilitate the correct interpretation of vectors' resistance to insecticides.

In parallel with the epidemiological studies being carried out on the impact of insecticide resistance on malaria transmission involving different vector species, and taking into account ecological conditions as regards the intensity of transmission, studies on the evasive behaviour of vectors should also continue. Some malaria vectors in South America, such as \textit{A. albimanus} and \textit{A. nunez-tovari}, have been studied in certain areas. Field investigations on these and other species should be carried out in different geographical areas. Such studies should provide guidance for the application of more appropriate methods and/or chemicals for malaria control and indicate particular lines of research to be carried out in laboratories on the mechanisms involved in the development and spreading of resistance in particular species of vectors.

4. Drug resistance monitoring

Resistance of human species of plasmodia to antimalaria drugs was detected from the mid-fifties onwards with pyrimethamine and proguanil and that of \textit{P. falciparum} to chloroquine from 1957 in Thailand and 1960 in Colombia. Initially, however, not much importance was given to drug resistance by most national services responsible for malaria control/eradication activities. This was probably due to the fact that too much reliance was placed on the use of insecticides on the one hand and on the other the \textit{in vivo} field test required long observation periods which, in certain instances, make the results questionable, since superinfection could not be entirely excluded. At the same time, no test kits were available for the \textit{in vitro} technique in the field and therefore this technique was only carried out by a few research workers. In the last five years, however, considerable efforts have been made in the study of the spreading of \textit{P. falciparum} resistance to chloroquine which has mainly been facilitated by an intensified training programme for nationals in the use of the \textit{in vitro} test and by the development of the WHO test kit.

A document entitled "Monitoring of drug sensitivity in \textit{Plasmodium falciparum}" prepared recently as an information paper will be distributed to the members of the RACMR and therefore I will not go into details on that subject here. However, it should be underlined that testing \textit{in vitro} or \textit{in vivo} does not represent monitoring \textit{per se}. What is required is the simultaneous controlled administration of other antimalarials with a view to establishing the most appropriate doses and regimens of those drugs available. This kind of study has been undertaken in Panama some years ago and should be done in other areas of the world. It is hoped, in the meantime, that the clinical trials on mefloquine in Brazil will yield results indicating the potency of this drug.
Another important aspect of monitoring is the identification of isolates from different geographical areas. The starch-gel electrophoresis technique should be used for this purpose, using a number of specific enzymes but "S" antigens could also be used as markers, as demonstrated by Wilson in Nigeria.

5. Operational problems linked with control methods

An analysis of the epidemiological situation in countries of the Americas shows that 33.2% of the total population originally exposed to the risk of malaria now lives in areas freed of the disease. This group consists of twelve countries in which there has been no resurgence of malaria, except for two outbreaks of P. malariæ infections, one in Trinidad and Tobago in 1966 and one in Grenada in 1978. In Trinidad and Tobago there were 30 cases detected and in Grenada a total of 58 cases were detected, confined to limited areas. Others in this group are: Chile, Cuba, Dominica, Guadeloupe, Jamaica, Martinique, Puerto Rico, St. Lucia, United States of America and United States Virgin Islands.

The second group comprises nine political units (Argentina, Belize, Costa Rica, French Guiana, Guyana, Panama, Paraguay and Dominican Republic) where transmission has been brought down to negligible levels. Within a population of 14.2 million in these countries (which represents 6.4% of the total population of the Americas exposed to the risk of malaria) 5 204 cases were reported in 1977 and 4 999 in 1978.

While malaria-free status in the first group can be relatively easily maintained with an adequate vigilance system, the classical methods of control with residual spraying and surveillance should bring the rate of transmission down even further in the second group. One has to bear in mind, however, that some of these countries, particularly those in Central America, are highly vulnerable and receptive.

The remaining 60% of the total population of the Americas originally exposed to the risk of infection lives in areas highly vulnerable and receptive to malaria, areas characterized by technical, operational, administrative and financial problems. Of these countries, Brazil, Ecuador, Mexico, Surinam and Venezuela are in a somewhat better position as the serious problems have been confined to limited areas, but programmes in Bolivia, Colombia, El Salvador, Guatemala, Haiti, Honduras, Nicaragua and Peru are encountering, over a large area, all the problems that can be met with in carrying out malaria control/eradication programmes i.e. multi-resistance of the vector(s) to insecticides, resistance of P. falciparum to 4-aminoquinolines, overuse to the point of misuse of insecticides in agriculture, inaccessibility of certain areas and all kinds of operational, logistic and financial problems.

Based on experience gained in the past 20 years, it is safe to say that malaria will still be a problem for quite some time to come. For this reason one must be very careful in selecting control methods. For example, the exclusive reliance even on new insecticides, particularly in Central America, may lead nowhere in the long run. In these areas, especially those on the Pacific Ocean side, our approach should be primarily to explore environmental
methods and supplement them at specific periods of the year by insecticides. In other words, an adequate balance must be found by applying source-reduction methods through environmental management, application of insecticides and pressure exercised on the parasite reservoir through antimalaria drugs. While there is no general prescription which would be valid for all situations, if the principle is accepted, national services should be in a position to establish field research activities with the object of demonstrating which of the three kinds of activities should be the basic measure and which would be supplementary under specific local ecological conditions.

Another group of field research activities should be directed towards finding out the most appropriate timing for vector control by chemicals. Admittedly, there are administrative difficulties hampering the application of house spraying during the shortest possible period of time just before the level of transmission rapidly increases. There are many examples, particularly in areas with seasonal transmission, in which there is a complete discordance between the season of intensive transmission and the timing of spraying operations. The frequency of these measures should also be reviewed in the light of local ecological conditions.

Migration of agricultural labour forces has long been one of the major constraints in achieving total coverage in space and time, again especially in Central America, not only because large segments of the population are moving around but also because of the temporary nature of human habitations, which makes the application of vector control measures impossible. To protect that population more imaginative approaches must be found for personal or family protection, depending on the environmental characteristics of the area. In addition, the use of slow release formulations of antimalaria drugs will have to be explored as soon as they are available.

Inaccessibility has also been a problem in several countries. However, although certain areas may be inaccessible to the malaria services, such areas are never completely hermetically closed and people are usually able to communicate at local level, thus reintroducing the parasite reservoir into the accessible areas under antimalaria operations. Here investigations should be carried out to develop better protective measures for the bordering accessible areas and for exploring flexible means of providing protection to the population of the inaccessible areas which would not be dependent on the actual physical presence of the malaria services in such areas.

Methods of epidemiological evaluation should also be reviewed and revised flexibly in accordance with the malaria rate in different areas. While for certain places the trend indicated by the slide positivity rate may be sufficient, in others more sophisticated methods of evaluation should be explored, including intensive surveillance, infant parasite rates or even seroepidemiological studies. In many instances there would be no need for special research but simply the application of existing methods of evaluation. We must, however, keep in mind the dynamics of malaria transmission and adjust our evaluation to the changing pattern of incidence and prevalence.

Finally, we should not forget that, without national will expressed through the political decision of a government and community participation, many efforts will not bear fruit. To mobilize the political leadership of
a country towards malaria control or eradication activities, the malaria service must review the impact of malaria on the socioeconomic development of the country and present a well-balanced summary of the situation as seen from both the medical (in terms of morbidity and mortality) and purely economic point of view.

In many areas, the population is hostile to the antimalaria measures applied by the national services. This is mainly due to the ignorance of the population on how malaria is being transmitted and partly due to the approach used by spraymen and surveillance agents in the course of their duties. For this reason field research will be required in many areas in order to establish locally acceptable and easily comprehensible forms of health education activities to enlist the participation of the community.

We all realise the complexity of the problems the malaria services have to face. However, some of these difficulties are not insurmountable but they do require thorough investigation to find a solution to them. With the cooperative efforts of the national malaria services, the scientific community and international organizations, it should be possible to make further progress and thus comply with the expressed aim of all governments of the Americas for the achievement of malaria eradication in that part of the world.
STRATEGIC PLAN FOR
APPLIED FIELD RESEARCH IN MALARIA

1 Developed by TDR/FIELDMAL-SWG
1) Strategic plan for applied field research on malaria

(1) To define the epidemiological & socioeconomic parameters related to malaria & their interaction

(2) To gain better knowledge of vector biology, ecology & behaviour

(3) To gain better knowledge of parasite species distribution & biology

(4) To gain better knowledge of malaria immunity

(5) To gain better knowledge of human behaviour and attitudes related to malaria

(6) To gain better knowledge of the structures & functions of health, manpower development & malaria services

(7) To make the best use of environmental control methods

(8) To make the best use of biological control of malaria vectors

(9) To make the best use of insecticides

(10) To make the best use of genetic control of vectors

(11) To make the best use of drugs both in the individual & the community

(12) To involve the community in malaria control activities

(13) To make the best use of personal protection methods against the vectors

(14) To make cost-effective combinations of methods

(15) Adapt the malaria surveillance methodology to different circumstances

(16) To develop planning methodologies

(17) Rationalize the planning, organization & management of malaria control

(18) To improve the methodology of training

(19) To achieve a better control of malaria
2) To gain better knowledge of vector biology, ecology and behaviour

(1) Define vector related malaria control problems

(2) Study of the behavioural differences of species complexes

(3) Study the behavioural characteristics of elusive vectors

(4) Determine the diagnostic dosage and interpret data on insecticide resistance

(5) Determine chromosomal characteristics of sibling species

(6) Determine electrophoretic enzyme patterns of sibling species

(7) Determine morphological characteristics of sibling species if any

(8) Assess their epidemiological significance

(9) Determine the geographical distribution of resistance and cross resistance

(10) Determine the geographical distribution of sibling species

(11) Determine the vectorial importance of vector sibling species

(12) Vector susceptibility to plasmodium strains

(13) Study the dynamics of insecticide resistance and cross resistance

(14) Determine the impact of agricultural use of insecticides on resistance in vectors

(15) To gain better knowledge of vector biology ecology and behaviour

Inputs from VBG: Develop sampling methods and techniques
Study natural habitats and life activities
Study vector biology
Study vector genetics
Develop new identification methods
Improve colonization methods
3) To gain better knowledge of parasite species distribution and biology

(1) Define parasite control problems

(2) Assess methods for sensitivity testing, study their significance and monitor their results

(3) Training in sensitivity testing

(4) Assess newly developed techniques of strain characterization of plasmodia

(5) Assess the status of sensitivity of human infections to antimalarial drugs and determine the significance of testing (a) in vivo (b) in vitro production of sensitivity test kits

(6) Assessment of sensitivity of Anopheles spp to plasmodia

(7) Gain better knowledge of parasite species distribution and biology

Inputs from CHEMM:

- Development of micro method for in vitro testing
- Dissemination of information on drug trials
- Development of techniques for strain characterization
4) To gain better knowledge of malaria immunity

(1) Assessment of immunological status of human populations as related to malaria control

(2) Field assessment of results of immunological methods

Inputs from INMEL

Development of immunodiagnostic tests
Improvement and standardization of established tests
Immunopathological studies
Studies of mechanisms of immunity and immunoevasion

Inputs from EPID

Study of epidemiological significance of immunodiagnostic tests
5) To gain better knowledge of human behaviour
and attitudes related to malaria

(2) To identify groups within different socio-economic
strata with specific beliefs and behaviour

(1) Identify beliefs and behaviour patterns related to malaria

(3) To identify groups within the health organization with specific attitudes towards malaria

(4) To assess the degree of interest of each group in malaria and its control

(5) To evolve strategies for health education programmes

Inputs from SER

Selection among existing methods for the assessment of beliefs, attitudes and motivation related to health problems
6) To gain better knowledge of the structures and functions of health care delivery systems that could be involved in malaria control.

(1) To describe the health manpower development programme, the health care delivery systems including the general health service, primary health care delivery systems, the malaria service and possible multisectorial collaboration.

(2) To define the relationship between the health care delivery systems, the health manpower development and the anti-malaria activities.

(3) To define the role of multisectorial approach and assess their impact on malaria control.

(4) To evaluate the efficiency, including the costs of different types of organized anti-malaria activities.

Input from SER and/or other relevant Units.
7) To make the best use of environmental control methods

(1) Review past experience in malaria control with engineering and naturalistic methods

(2) Study the characteristics of areas suitable for application of engineering and naturalistic methods including siting of human settlements

(3) Collect baseline data in selected areas for field trials

(4) Plan and implement field trials

(5) Assess the results of field trials and prepare guidelines for engineering and naturalistic control of malaria
8) To make the best use of biological control of malaria vectors

(1) Select the predators, parasites and other biological control agents suitable for malaria vector control

(2) Devise or improve methods of rearing, culture and mass production of larvivorous fish

(3) Study the effects of predators, parasites and other biological control agents on the environment

(4) Assess the methodology of application and of evaluation of various biological control agents

(5) Carry out field trials on malaria vector control by various biological agents

(6) Assess the results of field trials and prepare guidelines for biological control of malaria vectors

Inputs from BCV

To study parasites and other biological control agents for vector control

To study the ecological effects of biological vector control methods
9) To make the best use of insecticides

(1) Select the most promising, safe and effective insecticides

(2) Measure the impact of existing insecticides with entomological and parasitological parameters including adverse effects on elusive vectors

(3) Trials with newly developed insecticides and formulations

(4) Trials on different methods of insecticide application indoors and outdoors

(5) Improvement of the equipment dosage, formulation and application of insecticides and safety measure

Input from VBC

To develop new insecticides, formulations, dosages and application methodologies.
To study the toxicity of insecticides and their effects on the general ecology
To develop approaches towards overcoming insecticide resistance
10) To make the best use of genetic control of vectors

(1) Carry out field trials of genetic control methods if and when these become applicable

(2) Prepare guidelines for genetic control of vectors

Inputs from VBC

Developing methods for genetic control of vectors
11) To make the best use of drugs both in the individual and the community

(1) Identify problems arising from use of drugs in malaria control

(2) Assess the efficacy of drug regimens on malaria

(3) Study the side effects of antimalarials

(4) Assess the interaction of immunity and the use of antimalarials

(5) Monitor the sensitivity of parasites to antimalarials

(6) Select appropriate drug regimens

(7) Develop approaches to overcome parasite resistance to antimalarials

(8) Prepare guidelines for rational use of antimalarials

Inputs from CHEMAL

Testing new drugs
Improving drug regimens

Input from WHO

Develop methods for the assessment of immunity levels
12) To involve the community in malaria control activities

(1) Identify areas of participation/intervention by the community in malaria control

(2) Assess the potential for the participation of the community at various levels

(3) Identify factors producing resistance at various levels of the community

(4) Develop approaches to overcome resistance of the community, including health education

(5) Mobilize the community for malaria control

(6) Evaluate the extent of participation and its effects on malaria control

Inputs from SER

Selection of methods for assessment of knowledge, attitudes, behaviour and participation potential, and for community mobilization
13) To make the best use of personal protection methods against the vector

- (1) Review experience and literature in the use of personal protection methods against the vectors
- (2) To select methods and areas, and plan field trials
- (3) To carry out field trials with various methods
- (4) To evaluate the impact on man/vector contact and malaria incidence
To make cost/effective combinations of methods

1. Select areas for trials
2. Collect baseline data
3. Select mathematical models of malaria dynamics as appropriate
4. Simulate on the model the effect on malaria of single and combined control measures
5. Following limited trials to compare expectations with outcome
6. Calculate the costing of alternative combinations
7. Select the most cost/effective combination

Input from EPID
- Mathematical modelling
- Evaluation methodologies
15) Adapt the malaria surveillance methodology to different circumstances

(1) Identify goals of epidemiological surveillance in various programmes and situations

(2) Refine methods to measure factors having a bearing on malaria dynamics

(3) Identify information requirements at different levels

(4) Refine diagnostic techniques (clinical, parasitological and serological) and treatment procedures

(5) Assess improved methods of collection, processing, and flow of information

(6) Assess organisational patterns of surveillance/vigilance systems

Input from EPID

Biostatistics and data processing

Input from TMAL

Standardization of immunological tests
To develop planning methodologies

1. Review the situation in various countries regarding programme planning
2. Prepare draft research guidelines for programme planning
3. Test guidelines in different situations
4. Areas without malaria control
5. Areas with eradication control programmes which need re-orientation
6. Areas freed from malaria to prevent re-introduction of the disease
7. Areas with epidemic potential including malaria-free areas
8. To develop planning methodologies

N.B. This is a multidisciplinary action to be coordinated by MAP.
17) Rationalize the planning, organization and management of malaria control.²

(1) Identify national services, departments and responsible persons with an interest in malaria control

(2) Train national personnel in planning methodologies

(3) Analyze national problems

(4) Define goals and strategies

(5) Organize or re-structure the national malaria services

(6) Build information systems

(7) Promote training in malaria management

² NB Primary responsibility of MAP with government services.
18) To improve the training methodology

- Study the functions of personnel in malaria research and control
- Assess the requirements of trained personnel
- Prepare an inventory of research and training institutions and assess their capabilities
- Promote the development of training programmes
- Train the teachers and research workers
- Integrate research methodology in the current training curricula
- Organise and hold meetings for training in field research

Inputs from RSG:
- Institution strengthening
- Developing research and training capabilities

Note: Primary responsibility if MAP with government health services

NB Though the input of RSG is placed on this block, it is realised that this input may be required at a much earlier stage, in fact already at stage No.1 and as a continuing activity.
ANNEX II

CRITERIA FOR ASSIGNING PRIORITIES

A list of general criteria for the allocation of priorities was adopted:

1. In the first instance the problems, and the research projects aiming at their solution, could be rated according to the expected impact on malaria mortality and morbidity.

2. The duration, the lasting results, expected in a given research field, represents the second group of priority criteria. The definitive solutions should receive higher priorities.

3. The third category of criteria would include:
   (i) the feasibility;
   (ii) the duration;
   (iii) the cost.

of research projects. It should be correlated to the type and development phase of malaria activity in a given area, to the personnel, the equipment and other prerequisites, as well as to the time needed to solve the problems encountered.

4. Finally, the general policy of national governments as well as that of WHO should also be considered when defining the priorities of a given research topic. Investigations which will facilitate the start of a Malaria Action Programme or which will also promote the work in a larger area (region, world), or in other fields of interest to mankind, should be rated higher. In this context, consideration should be given to the proposed levels of central and peripheral interest.

It was recognized that these criteria would not be universally useful, but would form a basis for some comparability in the allocation of priorities among the several disciplines represented in the Strategic Plan.

In assigning priorities the following definitions were utilized:

Priority A: Projects to be implemented at once, if possible.
Priority B: Projects that may be postponed for a year, although great efforts should be made to implement them sooner.
Priority C: Important projects, to be sponsored as funds become available.

It was noted that these priorities also may not be universally applicable, perhaps differing in different regions or countries and in different epidemiologic situations.
The SWG was provided with a summary of the objectives, strategies and approaches of MAP towards global malaria control, with special attention to research topics which are essential to the success of such control programmes and which require coordinated action.

The objectives of MAP are to promote at national level the elaboration of realistic plans, based on endemo-epidemiology of malaria and the manpower and financial resources available in affected countries, with the aim of preventing/reducing mortality, shortening the duration of the disease, reducing its morbidity and prevalence and eradicating it whenever possible. In view of existing technical, operational and administrative problems, as well as the limitations of the current technology, it is considered that research must be an integral part of any malaria control/eradication programme. Further, at all levels the orientation of antimalaria activities should be based on field studies, keeping in mind at all times two principles, i.e. operational flexibility and epidemiologic approach. Appropriate programmes of research within the scope of the FİEİDMAL/SWG can satisfy many of the research needs associated with the MAP objectives. These would include thorough investigation of mechanisms for use of chemotherapeutic agents in areas where this may be the only appropriate measure. In other areas, where transmission still exists after many years of control or eradication effort, epidemiologic research will be needed to develop or refine effective approaches to control. This will include careful studies of entomologic, parasitologic, immunologic, environmental, socioeconomic and other factors which may have been responsible for continued transmission. In promoting this effort MAP will cooperate closely with the WHO Regional Offices and relevant Divisions on a global level, and provide a resource for coordination of research objectives of FİEİDMAL.

MAP has prepared a strategic plan for the implementation of its Programme, indicating in a logical sequence, the different actions that have to be taken in order to attain its objectives. This plan is included in the following tables:

Table 3: Malaria control strategy with the four main objectives:

<table>
<thead>
<tr>
<th>Level 1:</th>
<th>Reduction of mortality due to malaria and shortening the duration of illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 2:</td>
<td>Reduction/prevention of mortality and reduction of morbidity due to malaria</td>
</tr>
<tr>
<td>Level 3:</td>
<td>Reduction/prevention of mortality due to malaria, reduction of morbidity and of prevalence of the disease</td>
</tr>
<tr>
<td>Level 4:</td>
<td>Eradication of malaria</td>
</tr>
</tbody>
</table>
One or more of these can be selected in the same country according to political decisions and constraints.

Table 4: Administrative and political sine qua non conditions for the implementation of the strategy.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Expression of national will through political decisions for support</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Decision by the Government for long-term support</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Malaria control must be made an integral part of health programmes</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>The participation of the community should be secured</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Main lines of action.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control of epidemics, prevention of malaria spreading in malaria free areas</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Development of long-term programmes</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Promotion of training</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Promotion of basic and applied research</td>
<td></td>
</tr>
</tbody>
</table>
ROLE OF THE MALARIA ACTION PROGRAMME IN THE FRAMEWORK OF THE STRATEGY "HEALTH FOR ALL BY THE YEAR 2000"
MONITORING OF DRUG SENSITIVITY IN PLASMODIUM FALCIPARUM

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1 This paper has been prepared under the auspices of the Malaria Action Programme through contributions of Dr T. Lepes, Dr L. Molineaux and Dr W. H. Wernsdorfer.

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1. **Introduction**

It has long been observed that malaria parasite infections of *Plasmodium falciparum*, *P. vivax* and *P. malariae*, contracted in different geographical areas, provide a variety of clinical and other features, indicating the possible existence of different strains. In the first five decades of this century, scientists investigated the following features: possible morphological differences, observations which had been abandoned subsequently as they had not been found consistent; the incubation and duration of latency, particularly in relation to *P. falciparum*; relapsing pattern, pronounced in *P. vivax* infections; prolonged incubation period (also in *P. vivax* infections); response to treatment with quinine; immunological differences in all species in relation to superinfection and ability of a proven vector to support the sporogonic cycle of isolates of plasmodia from different geographical areas.

Most of the features of plasmodia mentioned above are epidemiologically important, some locally, others globally in relation to the possible spread of the disease or its introduction, or re-introduction, into an area already freed from malaria. It is understandable, therefore, that many attempts have been made in the past to develop a methodology for strain differentiation of plasmodia. While no such technique exists as yet, new biochemical markers have been identified which will certainly prove valuable, at least as far as certain aspects of strain characteristics are concerned.

As this paper deals with the monitoring of the sensitivity of *P. falciparum* to antimalarial drugs, it will mainly concentrate on features of plasmodia conditioning their response to these drugs.

Resistance of plasmodia to common antimalarials was first recorded by Neiva and by Nocht & Werner in 1910 in Brazil in relation to resistance of *P. falciparum* to quinine. In 1932, James, Nicol & Shute, working on isolates of *P. falciparum* from Italy (Rome) and India, demonstrated that the isolates from Rome required a treatment dosage of quinine eight times superior to that required for the isolates from India.

Regarding the newer compounds, although reduced sensitivity of *P. vivax* to pyrimethamine and/or proguanil has been reported from some countries in Asia and East Africa, and resistance confirmed in Malaysia, resistance of *P. falciparum* to pyrimethamine is widespread and is developing rapidly in areas where it is being administered alone on a large scale.

However, the resistance of *P. falciparum* to pyrimethamine is well understood and for many years now the recommendations insist that it should never be used alone, but only in combination with either chloroquine or a long-acting sulfonamide, mainly sulfadoxine. On the other hand, resistance of *P. falciparum* to chloroquine, first reported from Thailand in 1957 and later on from Colombia in 1966, has since spread over large areas in South-East Asia and Latin America. As chloroquine is considered to be one of the most efficient drugs, equally valuable for clinical treatment and for chemoprophylaxis, the resistance of *P. falciparum* has most important operational implications. This is the reason why the present programme of drug monitoring in malaria control programmes is based mainly on *P. falciparum* sensitivity/resistance to this drug.

2. **Definition and grading of drug resistance**

Drug resistance in malaria has been defined by a WHO scientific group on chemotherapy of malaria as the "ability of a parasite strain to survive and/or 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".

Between complete sensitivity and complete resistance, different degrees of tolerance can be observed, ranging from slow clearance of parasitaemia to temporary clearance and reappearance of the parasite, as well as instances where there has been no effect whatsoever on the parasitaemia. Consequently an arbitrary grading system has been established as follows:
S = for isolates sensitive (i.e. clearance of asexual parasites is obtained within seven days).

R I = clearance of asexual parasitaemia within seven days, followed by recrudescence.

R II = marked reduction of asexual parasitaemia but no clearance.

R III = no marked reduction of asexual parasitaemia.

3. Objectives

The ultimate objective of the programme is to secure the use of those antimalarial drugs, either individually or in combination, to which species of plasmodia, particularly *P. falciparum*, respond as expected when an optimal dose is administered.

Our main concern at this stage is the sensitivity of *P. falciparum* to chloroquine and other 4-aminoquinolines.

3.1 Major objectives

Detailed objectives of the programme can be summarized as follows:

(a) to assess the global status of *P. falciparum* sensitivity to chloroquine and other 4-aminoquinolines by *in vivo* and *in vitro* techniques, thereby establishing the current geographical distribution, prevalence and degree of resistance to these antimalarials;

(b) to assess, possibly on a global scale, the *in vivo* and *in vitro* response of *P. falciparum* to antimalarial drugs, other than 4-aminoquinolines, alone or in combination, to facilitate the administration of the most effective compounds;

(c) to develop systems of operational measures aimed at preventing the propagation of *P. falciparum* resistance to 4-aminoquinolines and, whenever possible, suppress malaria transmission in foci with resistant populations of this parasite.

3.2 Complementary objectives

In addition to the above, the programme will aim at characterizing different isolates of *P. falciparum* by identifying a certain number of specific enzymes synthesized by the parasite, to facilitate the prediction of the spreading of resistance on the one hand and on the other the opening up of further possibilities for investigating the vector-parasite relationship. This latter type of research would be particularly important in clarifying the ability of different proven vectors to support the sporogonic cycle of development of plasmodia strains isolated from different geographical areas.

4. Methods for testing drug sensitivity

4.1 In vivo tests

In the absence of simple, widely practicable methods for a procedure in accordance with the definition of drug resistance (section 2), *in vivo* tests were introduced which allow the observation of drug response in infected patients without the need for drug profile studies. These tests were primarily devised for an assessment of the blood schizontocidal effect of 4-aminoquinolines in *P. falciparum* infections. The WHO Standard Field Test consists of the administration of 25 mg chloroquine base per kilogram of body weight over three days, with a seven-day observation period (WHO, 1973). The observation period may be extended to 28 days ("extended test", WHO, 1973). During the whole test period blood slides are examined daily. Subjects included in the test should not have received antimalarial drugs during the preceding four weeks.
The results are to be interpreted as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disappearance of asexual parasites by day 6 (day 0 = first day of treatment) and no reappearance of asexual parasites by day 28</td>
<td>Sensitive = S</td>
</tr>
<tr>
<td>Disappearance of asexual parasites by day 6, but reappearance within days 7-28 (reinfection excluded)</td>
<td>Resistant, grade I = R I</td>
</tr>
<tr>
<td>Reduction of asexual parasites to 25% or less of the pre-test level within the first 28 hours of treatment, but no complete disappearance of asexual parasites by day 6</td>
<td>Resistant, grade II = R II</td>
</tr>
<tr>
<td>Maintenance of asexual parasitaemia at levels higher than 25% of the pre-treatment level within the first 48 hours of treatment</td>
<td>Resistant, grade III = R III</td>
</tr>
</tbody>
</table>

The Standard Field Test differentiates only between R II and R III on the one side, and S and R I on the other. R I responses can only be recognized in the extended (28 day) test.

The clinical tests, although indispensable for many purposes, have several major disadvantages:

(a) The relatively long, daily observation is difficult and costly to maintain in practice.

(b) Observation under conditions excluding reinfection, thus validating R I responses, may not always be possible in many areas.

(c) The test is only applicable to drugs or their combinations from which radical cure can be expected, e.g. 4-aminoquinolines, mefloquine and the combination of sulfadoxine and pyrimethamine. They are applicable neither to drugs such as pyrimethamine or quinine which are usually not employed in monotherapy nor to proguanil which is only used for suppression.

(d) Test results may occasionally be influenced by metabolic peculiarities of the patient or by pathologically accelerated gastro-intestinal passage.

(e) Relative immunity is known to blur results of the in vivo test, especially with regard to the differentiation between S and R I.

(f) The in vivo test does not provide an objective measure of the drug sensitivity of P. falciparum.

The in vivo test, in the described form, is only applicable to infections with P. falciparum and P. malariae. By combining it with a course of primaquine (0.25 mg per kilogram of body weight daily for 14 days), it can be extended to cover P. vivax and P. ovale infections as well. The modified test may also be used to assess the tissue schizontocidal action of primaquine provided that the essential long-term observation can be carried out under conditions which preclude reinfection.
4.2  **In vitro tests**

The **in vitro** test described in 1968 by Rieckmann et al. uses the measurement of schizont maturation in short-term, non-continuous culture. It remained for one decade the sole **in vitro** field method. In 1978, Rieckmann et al. adapted the principles of continuous culture of *P. falciparum* (Trager & Jensen, 1976) to the conditions of microculture and used these for the assessment of drug sensitivity in a short-term test against chloroquine and mefloquine. This method starts essentially from synchronized parasite material such as is usually available from patients with *P. falciparum* infections.

Richards & Maples (1979) described a very practical laboratory method for sensitivity testing with material from asynchronous continuous culture. This method is applicable to a wide range of antimalarial drugs, including dihydrofolate reductase inhibitors, and opens up possibilities for screening of candidate antimalarials. Through a bioassay, it may also offer interesting possibilities for measuring growth inhibition of drug metabolites. These techniques work with parasite isolates which are adapted to **in vitro** culture; they do not necessarily represent the characteristics of the parasite population from which the isolate has been obtained (Chin & Collins, 1979) and are therefore not suitable as field tests.

4.2.1 **Standard in vitro test (macro-test)**

The method described in 1968 by Rieckmann et al. has subsequently been further developed by Rieckmann & Lopez Antunano (1971) and by Valera & Shute (1975). Based on these experiences, the World Health Organization has standardized both the test procedure and the material which is now globally available in the form of standard test kits (WHO, 1979).

(a) **Performance**

The test is carried out with patients' blood containing 1000-80 000 young trophozoites (rings) of *P. falciparum* per μl. The majority of rings should be past the earliest ring stage and look "fleshy". Blood with mixed infections or from patients having received antimalaria treatment within the preceding four weeks should be excluded. Urine analysis for 4-aminoquinolines and sulfonamides prior to the test is advisable.

For the test, at least 10 ml of blood are obtained through venipuncture. The blood is emptied into a sterile Erlenmeyer flask, 25-50 ml capacity, with glass beads. Defibrination is effected by slow, regular swirling of the flask for five minutes. Aliquots of 1 ml defibrinated blood are then added to the test vials, the following types of which are available:

<table>
<thead>
<tr>
<th>Control</th>
<th>5 mg glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>n-mol chloroquine + 5 mg glucose</td>
</tr>
<tr>
<td>0.50</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>0.75</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>1.00</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
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<tr>
<td>3.00</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>4.00</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
</tbody>
</table>

| 0.25    | n-mol mefloquine + 5 mg glucose |
| 0.50    | " " " " |
| 0.75    | " " " " |
| 1.00    | " " " " |
| 1.50    | " " " " |
| 2.00    | " " " " |
10 ml of venous blood usually yields 8 ml defibrinated blood, sufficient for eight test vials. The sequence of vials should be selected accordingly, e.g. for areas with suspected resistance to chloroquine: 2 controls; 0.5, 1.0, 1.5, 2.0, 3.0 and 0.25 n-mol chloroquine. In areas with presumably normal sensitivity to chloroquine, the following sequence would be used: 2 controls; 0.5, 1.0, 0.75, 0.25, 1.50 and 2.00 n-mol chloroquine. Excess blood can be used to test against the other drug concentrations. The sequence for the test with mefloquine is 2 controls; 0.5, 1.0, 0.75, 0.25, 1.50 and 2.0 n-mol mefloquine.

After addition of defibrinated blood, the vials are closed and carefully swirled so as to dissolve glucose and drug. The vials are then incubated for 24 hours at 38.5°C in a water-bath or an incubator. After interrupting incubation the contents of the vials are thoroughly and gently stirred up for mixing before preparing thick films from each vial, preferably a whole test series on one glass slide, according to a set sequence. After adequate drying, the slides are stained in saline Giemsa at pH 7.0-7.1.

(b) Evaluation

The number of pre-schizonts (three chromatin bodies and more) and of schizonts is counted against 300 leukocytes, in samples with low parasite density against 1000 leukocytes. The mean of the two controls serves as the basis (100%) for the evaluation of the readings from the drug vials which is to be carried out according to the formula

\[ a = \frac{z}{m} \times 100 \]

\( a = \% \) pre-schizonts and schizonts as related to controls

\( z = \) number of pre-schizonts and schizonts per 300 (1000) leukocytes in drug vial

\( m = \) mean number of pre-schizonts and schizonts per 300 (1000) leukocytes from both controls.

Usually there is diminishing schizont maturation at increasing drug concentrations. Sensitive isolates show complete inhibition of schizont maturation at 1.25 n-mol (or less) chloroquine or 1.00 n-mol (or less) mefloquine per ml defibrinated blood.

(c) Constraints

The in vitro standard test has marked limitations. The necessity of venipuncture and the required blood volume hamper the application of tests in infants, young children, anaemic and severely ill patients. The critical range of 1000-80 000 predominantly "fleshy" ring forms per μl further limits the feasibility of the test. Samples with less than 1000 parasites per μl are not easily readable. Schizont maturation usually does not take place in samples containing more than 80 000 parasites per μl, probably as a result of an accumulation of lactic acid. This test system does not use a true growth medium. The only form of growth promotion consists of the addition of 5 mg glucose per ml defibrinated blood. Even the controls often reflect signs of poor growth conditions such as morphological changes of parasites and erythrocytes, multivacuolation and pigment clumping.

(d) Correlation between in vitro and in vivo findings

Valera & Shute (1975) found a strong correlation between the results of the in vivo and in vitro tests carried out in Luzon, Philippines. All cases with complete inhibition of schizont maturation at 1.0 n-mol chloroquine/ml defibrinated blood showed also clinical parasitological S responses in the extended (28 day) field test. On the other hand, R I responses were recorded in all patients where at least 1.5 n-mol chloroquine/ml defibrinated blood was required for completely inhibiting schizont maturation in vitro. The patients were recently infected, non-immunes, a fact which may also account for the clear-cut results. Comparison of the regression lines (exponential function) of growth inhibition in both drug sensitive and drug resistant (R I) groups showed very clear differences.

Further investigations have shown that the critical drug dose is in the area between 1.00 and 1.25 n-mol chloroquine/ml defibrinated blood: isolates showing full growth inhibition
at 1.00 n-mol reflect also clinical sensitivity of *P. falciparum* to chloroquine; isolates showing schizont maturation at 1.25 n-mol are almost invariably associated with R responses in non-immunes.

The *in vitro* test permits the drawing and characterization of inhibition profiles and, within certain limits of confidence, also the estimate of specific EDs. Schizont maturation at 3.0 n-mol chloroquine/ml defibrinated blood is usually a sign of R-II or R-III responses, but in this respect the *in vitro* standard test does not allow reliable predictions as the various R responses are also strongly dependent on host-determined factors.

4.2.2 Micro-technique (micro-test)

Employing the elements of the candle jar/petri dish method of continuous *in vitro* culture (Trager & Jensen, 1976), Rieckmann et al. (1978) have described a micro-technique for a 24-hour drug sensitivity test in *P. falciparum* which they had used with *Aotus* blood. After validating the technique for use with human blood from patients in Thailand, 1978, some further evaluation and modification of the system has been carried out in Brazil, Colombia and Sudan (Lopez Antúmano & Wernsdorfer, 1979; Kouznetsov et al., 1979).

(a) Microculture plates

The test is performed on flat bottom tissue culture plates (8 x 12 wells) which are pre-dosed with the appropriate drug, chloroquine or mefloquine - for trial purposes plates were also produced with Debechin (4 aminobenzo(g)-quinoline), a new drug from the USSR. The chloroquine and mefloquine plates contain the drugs in ascending doses from 0 (control) to 32 p-mol or 16 p-mol respectively.

(b) Test procedure

Persons who have received 4-aminoquinolines within the last 14 days, or pyrimethamine and/or sulfonamides within the last 28 days, should be excluded from the test. The pre-selected patients should be subjected to a urine test for chloroquine and amodiaquine and, if so indicated, for sulfonamides and those with a positive test should be excluded.

Thick and thin blood films are taken from persons suspected of having malaria. The films are Giemsa or Romanovsky stained and examined for malaria parasites. The *in vitro* test should not be carried out on patients with mixed infections or with counts of less than 500 asexual forms of *P. falciparum* parasites/μl. The stage of development of the asexual forms should be counted and recorded as the percentage of small, medium and large rings, on the form provided.

When the patient is selected, one proceeds as follows:

(i) Restitution of growth medium: 0.9 ml dilution fluid is injected by means of a sterile tuberculin syringe into a vial with freeze-dried medium and the latter dissolved by shaking.

(ii) The rubber stopper of the medium vial is removed.

(iii) One hundred microlitres of blood from finger tip or earlobe (or toe in infants) is drawn in a sterile, anticoagulant-treated capillary and ejected in the vial containing the growth medium. The vial is closed with a new, sterile rubber stopper and gently agitated so as to suspend the blood cells. Preferably, the blood/medium mixture should be processed immediately; but, if need be, it could be kept for up to three hours before adding it to the plates. However, the mixture should be kept as near to 37°C as possible. Higher temperatures and cooling are to be avoided.

(iv) The seal strips on the appropriate rows of the chloroquine and mefloquine plates are removed.
(v) All wells of the appropriate columns of the chloroquine and mefloquine plates are dosed with 50 ul of the blood/medium mixture in a descending order (starting from well A at the top, ending at well H at the lower end of the plate), using the Eppendorf pipette with a sterile tip. Whenever a new concentration series (line A-H) is to be started, it is necessary to change the tip in order to avoid drug contamination of the control wells. While dosing with the blood/medium mixture, the latter should be gently agitated in order to maintain the blood cells in suspension.

(vi) The lid is to be placed on the micro-plate and the patient's reference number inscribed on the lid, using a glass-writing pencil.

(vii) After the blood/medium mixture has been added, the culture plate should be shaken gently for a few seconds to dissolve the drug deposits in the wells.

(viii) The culture plate is then placed in an airtight container (preferably a desiccator) with a paraffin candle. (Only pure paraffin candles should be used.) After the candle is lit, the container lid is partially replaced, leaving only a small opening. The lid is finally closed just before the flame goes out. If desiccators with a stop-cock are used, the cover can be firmly fitted with the cock in the open position. The stopcock is closed when the flame is about to go out. The airtight container is then placed in an incubator at 37-38°C and left there for 24 hours if the majority of rings were large or medium size, or for 26 hours if the majority of rings were small.

Sealed waterbaths have also given very satisfactory results. Only types with slanting covers should be used, in order to avoid water dripping on the culture plates. The plates are placed above water level on a rack, the candle positioned on another and lit. The cover is put in place and sealed. Incubation at 37-38°C for 24 hours.

(ix) After incubation, two thick blood films are prepared from the contents of each well, after as much as possible of the supernatant has been removed by means of an ordinary capillary tube fitted with a bulb. The same capillary tube may be used for making the thick films, but a fresh tube must be used for each well.

(x) Thick films are stained for 30 minutes with Giemsa stain (2% solution in 7.1 pH phosphate buffer).

Since culture blood has a marked tendency to detach from the slide, it is advisable to keep the slides for two or three days prior to staining and use these slides for final reading.

If faster processing is desired, the slides are dried for two hours in the open air or in an incubator at 37°C (higher temperatures are to be avoided) and stained at pH 7.0-7.1 with a modified Romanovsky (Field) stain which allows the staining process to be completed within 10 minutes. The use of curved plates - slides facing down while staining - has given good results.

(xii) The schizonts are then counted against 200 asexual parasites and the results are calculated as follows:

<table>
<thead>
<tr>
<th>Controls</th>
<th>Samples with chloroquine or mefloquine</th>
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</thead>
<tbody>
<tr>
<td>Number of schizonts (i.e. parasites with more than two nuclei per 200 parasites after incubation)</td>
<td>Number of schizonts per 200 parasites after incubation</td>
</tr>
<tr>
<td>Control 1 from chloroquine plate</td>
<td>Control 2 from mefloquine plate</td>
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<tr>
<td>( K_1 )</td>
<td>( K_2 )</td>
</tr>
<tr>
<td>96</td>
<td>100</td>
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</tbody>
</table>
In cases of low parasitaemia, it is permissible to count against 100 parasites only.

(xii) The results are then recorded in the form (see Annex).

(xiii) If the growth in the controls is adequate and the parasites are only Plasmodium falciparum, the following may be concluded in the case of blood samples containing less than 90,000 parasites per μl:

- total inhibition of growth at 4.00 p-mol chloroquine per well indicates susceptibility to standard chloroquine treatment;
- growth at 5.7 p-mol chloroquine or more per well indicates resistance of F. falciparum to chloroquine;
- growth at 4.00 p-mol chloroquine per well, but inhibition at 5.7 p-mol may still be compatible with a satisfactory response to chloroquine;
- preliminary in vitro results with mefloquine in the micro-test indicate that a dose of 4.00 p-mol normally completely inhibits maturation of schizonts. This concentration of mefloquine may possibly not be sufficient to prevent schizogony when parasitaemia is high (>90,000 parasites/μl).

(c) Application

The in vitro micro-test is not meant to replace the in vivo assessment of the response to treatment with chloroquine. However, it provides a useful means for monitoring parasite sensitivity to drugs and is a convenient method for detecting the emergence of drug resistance.

The micro-test has a much wider range of application than the in vitro Standard Test (macro-test) since two series of tests, e.g. with chloroquine and mefloquine, can be run with 100 μl blood which can easily be obtained through finger-prick. The stage of development of the rings is not critical. Damage of the blood samples in transport is very rare since the blood is taken up in medium. There is also apparently no upper limit of parasitaemia at which the test can be applied - blood from patients very rarely exceeds 10% parasitaemia. However, reading and evaluation of the test may be impaired at parasite densities exceeding 90,000/μl blood. Growth conditions in the micro-test are better than in the macro-test. This is evident from the morphology of parasites from the control wells. However, these suitable growth conditions will lead to the bursting of schizonts and merozoite invasion of other erythrocytes when the incubation time is prolonged. This invalidates the test which is essentially based on the evaluation of the growth differential of originally synchronous parasite material.

The growth medium contains gentamycin. Previous experiments showed that this antibiotic - in the dose used - does not interfere with parasite growth, but it does sufficiently suppress the often unavoidable bacterial contamination in the short-term test.

Previous experience with full blood treated with anticoagulants such as heparin and EDTA indicates a serious interference with the staining characteristics. However, Giemsa staining of blood samples from the micro-test produces satisfactory results. Much of the anticoagulant is obviously removed with the supernatant and thus does not influence the staining.

5. Implementation

5.1 Training

The first interregional course on the assessment of drug response in falciparum malaria was held in Peninsular Malaysia in 1973. A major expansion of training started in 1977, when standard test kits for the in vitro (macro-) test had become available and the monitoring of the drug response of Plasmodium falciparum imperative. Between 1977 and 1979 some seven
Interregional, regional and subregional courses were held in Brazil, Colombia, El Salvador, Malaysia, Sudan, Tanzania and Thailand, training a total of over 100 parasitologists and senior laboratory technicians. Several national courses were held in countries of the American, South-East Asian and Western Pacific Regions of WHO, fulfilling the requirements of these regions. More training is required, in the Eastern Mediterranean and more particularly for the African Region, for which three regional courses are being prepared for 1980-1981.

While all training prior to 1980 was geared to the use of in vivo and in vitro standard (macro-) methods, the training in 1980/81 will also include the in vitro micro-method. Staff of the American, South-East Asian and Western Pacific Regions will also be trained in the micro-technique, using for this purpose regional or subregional meetings of the technical personnel involved in the monitoring of drug response. Such meetings are regularly being arranged for the exchange of information and the updating of technology and methodology.

5.2 Production and distribution of test kits

After standardizing the technique for the production of standard test kits at WHO headquarters, Geneva, the mass production of the standard (macro-) test kits has been transferred to the Central Laboratory, Malaria Eradication Programme, Manila, Philippines, which undertakes these activities under a contractual technical services agreement with the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (Applied Field Research on Malaria - FIELDMAL). The annual production of test kits (1969 and 1980) amounts to 200 kits A, 400 kits B and 200 kits C. Test kit A is the basic set for chloroquine; kit B and kit C are the replenishment kits for chloroquine and mefloquine. The present production of the Manila centre permits a test volume of up to 18,000 tests per year.

The quality control of the test kits, covering sterility, glucose- and chloroquine-content of the vials, is handled by the Central Laboratory, Bureau of Tropical Diseases, Center for Disease Control (CDC), Atlanta, on a monthly basis.

The distribution of the standard (macro-) test kits is being handled by the Malaria Unit, WHO Regional Office for the Western Pacific, according to requests from the regional offices, in coordination with WHO headquarters. A satisfactory stock situation permits the meeting of emergency requests.

The micro-test is still in the developmental stage. Distribution of this material is being effected by WHO headquarters and was hitherto restricted to the laboratories and services collaborating in the development of the test system.

5.3 Organization of field investigations

The field investigations are planned on a national basis by the responsible services, and integrated into the regional collaborative activities which pertain to the three major objectives under 3.1, in summary:

(a) baseline assessment and monitoring of chloroquine sensitivity in \textit{P. falciparum};

(b) baseline assessment and monitoring of sensitivity of \textit{P. falciparum} to drugs other than chloroquine;

(c) development, evaluation and broad application of containment measures.

Range and organization of field investigations largely depends on the incidence and prevalence of \textit{falciparum} malaria, and on already existing or threatening problems of drug resistance. The programme is essentially global in nature and has to strive at the best fulfilment of the above objectives.

5.3.1 Baseline assessment/monitoring of chloroquine sensitivity

While sampling can and should be done on a representative basis, it is realized that this is only feasible in areas with relatively high endemicity or during substantial epidemics.
These are rare conditions in most of the Asian and American malarious countries, where opportunistic sampling has to play the major role. Complete sampling of cases suspected of a resistant response to chloroquine is necessary in areas where *P. falciparum* had hitherto responded normally to the drug. Monitoring of chloroquine sensitivity can be reduced to a lower, yet statistically acceptable level in areas where both frequency and degree of chloroquine resistance are high.

It is realized that the blood volume to be taken for the *in vitro* standard (macro-) test precludes its wide use in young children and infants. This constraint is expected to be overcome with the micro-test.

### 5.3.2 Baseline assessment/monitoring, other drugs

For sampling the same conditions apply as mentioned under 5.3.1. Apart from assessing and monitoring the current response of *P. falciparum* to amodiaquine, quinine, pyrimethamine and sulfadoxine/pyrimethamine, it will be necessary to obtain a true baseline for mefloquine before this drug can be commercially introduced, and to monitor the appropriate response levels longitudinally in relation to the specific drug pressure. The same applies to any other candidate drugs reaching the stage of phase I-III clinical trials. While part of this work, as related to mefloquine, can already be undertaken with the available methods, *in vitro* testing with quinine and dihydrofolate reductase/pteridine synthetase inhibitors will have to await the introduction of the *in vitro* micro-method.

### 5.3.3 Containment studies and measures

Such studies and operations are of paramount importance to countries affected or threatened to be affected by the problem of drug-resistant *P. falciparum*. The orientation of these activities can be very different according to the actual local situation, such as:

- major, intensive malaria control/eradication measures, mainly through vector control, in areas with widespread or multifocal resistance;
- intensive focal and circumfocal eradication/control measures concerning smaller, isolated foci, mainly relying on vector control measures;
- surveillance/vigilance measures, if so indicated supported by vector control measures, in areas exposed to the importation of resistant *P. falciparum* infections.

In all of the above areas it will be necessary to attempt the fullest possible detection and appropriate treatment (including the use of gametocytocidals) of falciparum malaria cases.

Operations under 5.3.1 have been widely implemented in the American, South-East Asian and Western Pacific Regions; activities under 5.3.3 are currently being undertaken in the two latter Regions. Wider implementation of operations under 5.3.2 is largely dependent on the introduction of the *in vitro* micro-test which is expected for 1981.

Countries of the Eastern Mediterranean Region defined the scope and orientation of the regional collaborative studies only in November 1979; practical implementation on a large scale is planned for the end of 1980. No region-wide programme has as yet been defined in the African Region.

The organization and performance of the field studies are a national activity/responsibility. The WHO regional offices, in collaboration with WHO headquarters, undertake technical coordination, training and logistic back-up, and exchange of information.

### 5.4 Collection and analysis of data

#### 5.4.1 Justification for an ad hoc information system

An *ad hoc* information system concerned with the sensitivity of malaria parasites to antimalarial drugs serves the following purposes: (1) description of the distribution,
according to time and place, of the susceptibility status of the various species of malaria parasites to the various antimalarial drugs; (2) generation and/or testing of hypotheses explaining the above distribution in terms of possible causal factors (e.g. drug pressure, characteristics of resistant parasite strains, characteristics of vectors, human migrations); (3) guidance for action aimed at correcting the consequences of drug resistance (e.g. through selection of the most appropriate substitute drugs), or at reducing the prevalence and geographical distribution of drug-resistant strains (e.g. through the control of transmission), or to prevent the appearance or further extension of drug resistance (e.g. through a more judicious use of all control methods, through the application of special measures to migrants, etc.); (4) operational evaluation of the tests and their performance (e.g. savings versus information lost through reduction of the number of drug concentrations used; variability of test results as a guide for setting future sample sizes).

5.4.2 Justification for uniformity and centralization

For all four of the purposes listed above, uniform methods of collection and analysis of data are necessary. This does not prevent flexibility in the collection of additional data or in the performance of supplementary analyses. The required degree of uniformity will be reached more economically if the methods of collection and analysis of data are developed centrally, and their application decentralized. The four purposes listed are relevant both at the peripheral and at the central (global) level, and the ad hoc information system should be developed accordingly.

5.4.3 Status of implementation

Ever since the discovery of drug resistance in malaria parasites, WHO has been involved in collecting, processing, analysing, and distributing the relevant information, and in the search for corrective measures. The current effort to strengthen this activity results from the following factors:

(1) the increasing magnitude of the problem;
(2) the increasing utilization of drugs for the control of malaria;
(3) the availability of new diagnostic tools, the in vitro tests.

Recent developments towards the establishment of an ad hoc information system are the following:

(1) creation of a technical task force in WHO/Malaria Action Programme (MAP);
(2) consultations with regional offices, and with most of the scientists directly concerned inside and outside WHO;
(3) production, through several critical revisions, of a standard precoded record form for the results of in vitro macro- and micro-tests of the sensitivity of P. falciparum to chloroquine and mefloquine (see Annex);
(4) small-scale trials of the recording of test results on the new form;
(5) estimation of costs involved in printing the forms and in processing the information;
(6) allotment of a budget for launching the new system.

5.4.4 Epidemiological analysis and interpretation of findings

The analysis of the results will describe the composition of parasite populations according to their sensitivity to drugs, and study how their composition varies over time and between geographic locations. The study should include the parasite population of areas where no resistance has ever been demonstrated and/or where drug pressure has been negligible.
Areas where most parasites are highly resistant are also interesting, in particular if drug pressure is reduced. It is desirable to investigate samples that are (a) representative of the parasite population infecting a given human population; and (b) sufficiently large. In practice one should try to get as close to such sample quality as reasonably feasible.

The interpretation (explanation) of the findings will involve (a) correlation with the distribution in time and space of other variables (e.g., utilization of the same or related drugs, human migration, vector species, intensity of transmission, and immunity level of the human population); (b) comparison with the results of clinical and laboratory research, including research on non-human parasites (e.g., genetics of resistance; evolution of mixed sensitive and resistant infections; infectivity of various resistant strains for various vector species; possibility of selection for resistance in human parasites from different geographic areas; factors favouring or preventing selection for resistance). The clinical and laboratory research will suggest certain variables for study in the field; conversely, the epidemiological investigations will suggest certain laboratory and clinical investigations, and will help in the setting of priorities among such investigations.

Description and interpretation are justified by their contribution to action. That contribution is already obvious, i.e., for decisions re the choice and dosage of drugs for treatment and prophylaxis, decisions re the expected consumption and desirable production of various drugs, decisions re special efforts to reduce transmission. Further investigations aimed at a much more thorough understanding of drug resistance as an epidemiological phenomenon are, however, amply justified in practice, in order to orient decisions re tentative proposals for control (including, for example, using certain drugs in association while banning some others, or using drugs in alternation) and to imagine and develop new control methods or procedures. Decisions re control of the phenomenon of resistance would obviously be easier to take if our understanding of the phenomenon were such that we could design realistic simulations of the impact of various single or combined interventions. Exploratory simulations, based on the limited information available, would also assist in pinpointing some of the more serious gaps in our knowledge.

5.5 Parameters for expressing drug responses in in vitro tests

Past experience has shown that the values of ED$_{90}$ and ED$_{100}$ are of particular importance in areas with generally non-immune subjects. The same applies to non-immune patients ("sentinel cases") exposed to infections in areas with generally high endemicity and immunity. In the latter areas the values of ED$_{50}$ and ED$_{90}$ are of specific significance, the former reflecting particularly the broad response of the parasite population, the latter closely reflecting clinical response in semi-immune subjects.

Since many areas are subject to transition from a state of higher to one of lower endemicity, or vice versa, with the implicit changes of communal immunity, and, since the monitoring programme is global, it has been recommended to retain uniformly the ED$_{50}$, ED$_{90}$ and ED$_{100}$ as the key parameters of drug response in schizont maturation tests.

This applies to all drugs used in the appropriate test systems.

The ED$_{50}$, ED$_{90}$ and ED$_{100}$ can be read from the graphs of the individual regressions. Individual ED$_{50}$ and ED$_{90}$ are, of course, subject to a considerable margin of error, but evaluation and follow-up on community or larger geographical basis reduce error considerably and make longitudinal assessment (monitoring) quite precise and meaningful.

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1 The effective dose 90 (ED$_{90}$) refers to the drug quantity in a given blood volume which results in a 90% inhibition of schizont maturation as compared to the drug-free controls. Similarly, ED$_{50}$ and ED$_{100}$ denote the drug quantities which produce 50% and 100% (complete) inhibition of schizont maturation. The graded effective doses are expressed in substance concentration of the drug related to blood volume (according to the SI system).
6. Strain characterization

6.1 Principle

As indicated in the introduction, certain specific features of any species of plasmodia which were reproducible and stable have been considered in the past as characteristics of "races" or "strains". It was necessary, therefore, to look for genetic markers, persisting with relative stability in the plasmodia population, which could be used as identifying elements for any particular population of plasmodia.

Generally, it has been known for some time that genetic differences between microorganisms can be identified through variations in enzyme synthesis or DNA characteristics. Since the work done by Carter in 1970 on genetic characteristics of plasmodia identifying some enzymes in the *Plasmodium berghei* model, it has become possible to use enzyme electrophoresis as genetic markers of different isolates of human species of plasmodia. This technique will be used, therefore, to characterize different isolates.

It should be pointed out, however, that, while it may be correct to refer to "strains" in colloquial language, the term does not correspond to reality. For this reason we should use the term "isolate" which means a population of plasmodia collected from a single individual on one single occasion. In contrast with a "strain" which should be the population of plasmodia genetically homogenous, the isolate is not necessarily genetically homogenous. An isolate which has undergone laboratory passage should be called "line".

6.2 Sampling of isolates and enzyme identification

It is known that isolates of *Plasmodium falciparum* from various parts of the world differ in their biological characteristics, e.g. in the ability to infect a particular anopheline species. It is likely that these functional characteristics extend also to the ability/rapidity of producing drug-resistant mutants. Therefore it is essential to collect adequate samples of *P. falciparum* from all endemic areas around the world and to characterize them biologically. These studies used to be followed up by longitudinal monitoring since it was important to obtain an insight into parasite population dynamics, especially in relation to drug pressure. At present the biological characterization is limited to the identification, through starch gel electrophoresis, of the isoenzyme pattern of the following enzymes:

- glucose phosphate isomerase (GPI), EC 5.3.1.9
- phosphoglucone dehydrogenase (PGD), EC 1.1.1.43
- lactate dehydrogenase (LDH), EC 1.1.1.27
- glutamate dehydrogenase (GDH), EC 1.4.1.2
- adenosine deaminase (ADA), EC 3.5.4.17
- peptidase E, EC 3.4.21.16

These procedures require the continuous in vitro culture of *P. falciparum* as an essential tool. While these studies are serving primarily epidemiological purposes, especially in relation to drug sensitivity and malaria transmission, they should also produce a set of well characterized, biologically different isolates of *P. falciparum* from various parts of the world which could routinely be used in chemotherapeutic, immunological and basic biological research. Ideally, such isolates should be cloned - a relatively suitable technique by dilution has recently been developed at the WHO Collaborating Centre for Strain Identification, Edinburgh. The use of the standard isolates would assure the comparability of results obtained by different laboratories.

The sampling and biological characterization of isolates, as well as the maintenance and the distribution of standard isolates, require a network of collaborating centres (isolate banks). In addition to the centre in Edinburgh, a collaborating institute in Thailand could fulfill this role for Asia; another institute in Brazil may soon be able to do the same for South America. Other potential centres are in Atlanta, United States of America, and Shanghai, China.
6.3 Cryopreservation of isolates

During the last decade, and especially since the development of the technique for continuous cultivation of \textit{P. falciparum}, several methods have been developed for cryopreservation of malaria parasites. These methods were developed for various technical reasons, e.g. to facilitate the transport of parasites between laboratories and between the field and laboratory, and to bring human parasite material within the reach of laboratories outside the endemic areas.

In view of the varying results of different methods and of the importance of the subject for a wide range of malaria research, an informal consultation was held in February 1979 with the participation of scientists from CDC, Atlanta, Walter Reed Army Institute of Research, Washington, Biomedical Research Institute, Rockville, Naval Medical Research Institute, Bethesda, Gorgas Memorial Laboratory, Panama, and two staff members of RTI/MAP. The objectives of this meeting were a review of the present status of experience in the field of cryopreserving malaria parasites, the identification of lacunae in knowledge and the formulation of a research plan.

A review of the present status of research in cryopreservation of the erythrocytic stages indicated that:

(a) infection of laboratory animals with cryopreserved parasites of established laboratory strains was routinely possible;

(b) continuous cultivation of cryopreserved parasites obtained from established culture lines was possible;

(c) the viability of these parasites following cryopreservation was low. Ring stage parasites survive the procedure, but late trophozoites and schizont stages do not;

(d) major difficulties have been encountered when attempting both to infect laboratory animals and to establish cultures from strains of \textit{P. falciparum} isolated in the field;

(e) some of the methods presently developed are laborious and difficult to standardize and adapt for use in the field. Moreover, they present major logistic problems.

In view of the need to investigate and monitor the status of resistance to antimalarial drugs, particularly of \textit{P. falciparum} to chloroquine, there is an urgent need to develop techniques for the cryopreservation of strains in the field which will subsequently enable the continuous cultivation \textit{in vitro}. The maintenance of such strains \textit{in vitro} will allow their complete characterization by drug susceptibility, antigenic analysis (when techniques become available), and enzyme characterization. In addition, they are essential to the establishment of reference banks of malaria parasites of known characteristics, the establishment of which was also recommended by the Scientific Working Group on the Chemotherapy of Malaria at its second meeting.

In addition, a detailed comparative study of the various methods currently used for cryopreservation is required to optimize and standardize the conditions used, particularly in relation to the type of cryopreservant (DMSO, glycerol and sugar derivatives), rates of cooling and thawing, and types of apparatus used.

Following these lines, a major collaborative research project (TDR, Scientific Working Group on the Chemotherapy of Malaria) has been established with the Center for Disease Control, Atlanta, in 1979, also involving several of its overseas branches.

6.4 Collaborative laboratories

The technique of gel electrophoresis has been well described and, from that point of view, it could be applied on a large scale. However, at this stage, and taking into account the need for considerable preparatory work, there has been only one WHO collaborative centre...
nominated, i.e. Institute of Animal Genetics (Professor Beale), Protozoan Genetic Unit, University of Edinburgh, West Mains Road, Edinburgh EH9 3JN, United Kingdom. It is expected that another collaborative centre, possibly at the Chulalongkorn University, Department of Biology (Professor Sodsri Thaithong), which is already included in the network on strain characterization, will also be nominated.

At present the WHO Collaborative Centre is providing training for scientists of various nationalities interested in acquiring the technique, and is investigating different isolates of human plasmodia in the field. It also carries out research on the genetics of rodent plasmodia in relation to the location of mutations leading to resistance to different antimalarial drugs. The Centre has also undertaken the study of additional enzymes, other gel techniques, especially polyacrylamide and 2-dimensional electrophoresis of radioactively-labelled proteins (polyacrylamide and SDS gel).

The important study on strain characterization is at its initial stage. It has been motivated mainly for the purpose of predicting the spread of chloroquine resistance but, in view of the importance it may have in the epidemiology of malaria, through ability of specific strains to survive in the presence of different antimalarial drugs or in passing the sporogonic cycle in different vector species and strains, a considerable effort will be required to develop the technique itself and to identify more isolates of human plasmodia from different geographical areas.

7. **Further research required**

Further research should be stimulated in parallel, along two specific lines:

(a) development of (micro-) test systems for drugs other than chloroquine and mefloquine, i.e. pyrimethamine, proguanil, quinine, sulfadoxine, fansidar;

(b) strain characterization.

The latter aspect could be divided into:

(1) further studies on cryopreservation, with particular reference to genetic composition of isolates after different time intervals since their initial freezing. These studies will have to be carried out with laboratory animals and with in vitro cultivated parasites. This will require the nomination of additional collaborative centres;

(2) determination of additional important biological markers, especially those related to drug resistance, e.g. 7,8 dihydrofolate reductase (DHFR) and pteridine synthetase;

(3) identification of DNA properties through the measurement of DNA buoyant density and the determination of hybridization properties;

(4) research aiming at improving methods of assays, such as the use of polyacrylamide electrophoresis which may require less material for the test;

(5) introduction of enzymes other than those already being investigated;

(6) research on antigenic markers which would complement the biological characterization of isolates as, for example, S-antigens as demonstrated by Wilson in Nigeria. Development in this direction also became feasible through the production of monoclonal antibodies from hybridomas.

As can be seen, the programme of monitoring of *P. falciparum*’s sensitivity to drugs is a very complex one. However, this programme is conceived so as to provide a wide background not only on the geographical distribution of resistant *P. falciparum* but also to facilitate its true monitoring. At the same time studies related to strain characterization will facilitate in part our comprehension of the mode of action of drugs and also serve as a basis for more rational development of new drugs. Of equal importance to the laboratory studies is the training of nationals in countries with endemic malaria and the evaluation of the results and their correct interpretation, enabling the responsible services to fully appreciate the operational impact of drug resistance in plasmodia.
RESPONSE OF P. FALCIPARUM TO CHLORQUINE AND MEFLOQUINE (IN VITRO-TEST)

A COUNTRY AND PLACE OF TEST

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B COUNTRY AND PLACE INFECTION PROBABLY CONTRACTED

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D INCUBATION

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<thead>
<tr>
<th>Date</th>
<th>Duration (hours):</th>
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E PATIENT

<table>
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<tr>
<th>Sex</th>
<th>Age</th>
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<tbody>
<tr>
<td>M/F</td>
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</table>

F REASON FOR SCREENING

- 1 = Resistant or suspected resistant case
- 2 = Collateral case of resistant or suspected resistant case
- 3 = Resist. in area of origin
- 4 = Routine monitoring
- 5 = Resist. in adjacent area
- 6 = Resist. in other rel. area

G SAMPLE

- 1 = General pop.
- 2 = Labour force
- 3 = Infected
- 4 = Outpatient
- 5 = Migrant labour

H DRUG TAKEN DURING LAST 2 WEEKS

| History: | Any anti-malarial drug taken? | Yes/No | | | | | |
|----------|-----------------------------|-------|---|---|---|---|

I PRE-CULTURE SLIDE EXAM.

<table>
<thead>
<tr>
<th>No. annual Pl/mmc3 blood:</th>
<th>Number counted:</th>
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J RESULT OF MACRO-TEST

<table>
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<tr>
<th>Chloroquine 48h batch n.</th>
<th>Mefloquine 48h batch n.</th>
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K RESULT OF MICRO-TEST

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<tr>
<th>Chloroquine plate batch n.</th>
<th>Mefloquine plate batch n.</th>
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L Where the slides referred for checking?  - Yes  - No

M Did the patient travel, and where (during the last 12 months)?

N Conclusion:
SUMMARY OF SCIENTIFIC PROGRESS IN THE FIELD OF MALARIA PUBLISHED DURING THE LAST FIVE YEARS

Information paper jointly prepared by the
Malaria Action Programme
and
Division of Vector Biology and Control

1980

Contributors

Dr J. F. Copplestone  Dr P. Pal
Dr R. Darwish       Dr C. Pant
Dr J. Hamon         Dr C. Quélennecc
Mrs M. King         Mr H. Rafatjah
Mr C. Kuo           Dr N. Rishikesh
Dr T. Lepes         Miss J. Robertson
Dr L. Martinez      Dr P. I. Trigg
Dr L. Molineaux     Dr W. H. Wernsdorfer
Dr E. Onori

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INTRODUCTION

In the hundred years since Laveran's discovery of plasmodia as a causative agent of malaria, the subject has continued to be of much concern to scientists and health administrators. The various aspects of malaria, as a disease, its causative agents, the vector, its epidemiology and control, have all raised particular interest at different periods. Initially the morphology and biological life cycle of the parasite and vector, including basic epidemiology of the disease, were given priority. Later, it was the development of synthetic antimalarial drugs and studies on immune phenomena which featured in the specialized medical journals.

The relationship between the host and the parasite is so complex that it requires further research. Being true parasites, plasmodia are, of course, unable to complete their metabolism without utilizing the metabolic processes of the host cells and tissues. The following examples of scientific progress, and the period of time which elapsed between each event, clearly illustrate the complexity of this relationship. The first attempt to cultivate human plasmodia in vitro was made in 1912 by Bass with some success but no further progress was achieved until 1940 when Trager made an attempt with Plasmodium lophurae. It was only in 1976 that Trager and Jensen were able to demonstrate the procedure for continuous in vitro cultivation of P. falciparum. Golgi, in 1886, and Metchnikoff, in 1887, were the first to observe malaria parasites in macrophages but, even today, we do not yet fully understand the cell-mediated immunity in malaria. Sotiriades made the first attempt at immunization against malaria in 1915 when he used serum taken from a convalescent patient on acutely ill patients and obtained clinical improvement. In 1942 Mulligan tried to use irradiated sporozoites in a P. gallinaceum model, producing some evidence of protection. However, it was only from 1967 onwards that intensified research on the development of an immunizing agent against malaria began to make good progress. The development of a vaccine, if it finally materializes, will take some time.

In the field of chemotherapy similar difficulties have been encountered. In the United States alone, over 250,000 compounds have been screened and tested in the last 15 years, from which four groups of molecules have emerged as potential candidate antimalarials. Among these, the testing of mefloquine has made the most advance.

Studies on biology of plasmodia, their metabolism, in vitro cultivation and manipulation of their different developmental stages may not have a direct impact on malaria control. Nevertheless, this type of research is essential for understanding the intricate relationship between the parasite and the host cells and tissues. At the same time, studies on biology of plasmodia provide the basis for further development of chemotherapy and immunity, including immunization in malarial infection, and in both aspects as regards the methodology applicable and the rational approach in relation to the development of antimalarial drugs and immunizing agents. Under the aegis of the Special Programme for Research and Training in Tropical Diseases, and in cooperation with USAID, a consultation between many scientists working on related subjects took place in 1977. Additional details may be found in the WHO Bulletin, 1977, Vol. 55, Nos. 2-3, pp. 124-430 and 1979, Vol. 57, Supplement 1.
In the development of methods and tools for the control of the disease there have been some new chemicals for residual house spraying, or other kinds of application, or for larviciding, but none of these can solve all the problems of malaria control in different ecological conditions nor can we expect immediate answers.

However, efforts are continuing and there is no place for undue pessimism. The Organization has closely followed scientific progress and stimulated malaria research for more than 20 years. In fact, this close collaboration within the worldwide scientific community has facilitated the relatively easy development of the Special programme for Research and Training in Tropical Diseases.

In spite of the fact that, so far, no vaccine has been developed and not many new drugs or insecticides have been made available, tremendous progress has been achieved in our understanding of different phenomena and in the development of new techniques for research. The considerable advance made within the biological sciences (cell biology, membrane biology, molecular biology, biochemistry and immunology) has assisted the application of many techniques in research on the malaria parasite, the vector and the host. As the articles published on various aspects of the disease are dispersed throughout the many different medical and biology periodicals and are thus not always accessible to those interested, a summary of scientific progress published in the last five years has been prepared. It is hoped that the health administrators and technicians interested in the subject will gather from this data information on how research in the field of malaria is oriented, on the progress made so far and on what the world may expect from the colossal work the international scientific community is carrying out.

1. THE PARASITE

1.1 Life cycle

A considerable amount of new knowledge about the parasite's life cycle has become available in recent years. Since it concerns almost all phases of the cycle, a full, up-to-date description of the latter is given below. For the sake of clarity, the citation of earlier literature is included in the description.

The sporozoite, upon inoculation by an infected mosquito, reaches the blood circulation in which it is transported to the target site or its vicinity. Possibly guided by chemotaxis and recognizing its target, it leaves the capillary lumen and enters the host cell, either directly or possibly through the intermediary of another cell. The host cell can be a hepatocyte (as in mammalian plasmodia) or reticuloendothelial cells (as in avian plasmodia). In no case do sporozoites enter red blood cells. Fairley's observations (1945) on \textit{P. vivax} showed that sporozoites vanished from the circulating blood within 30 minutes of inoculation. The sporozoite's entry into the host cell results in a drastic morphological change in the parasite which now appears round or oval and contains a chromatin nucleus surrounded by cytoplasm. In the course of the ensuing exoerythrocytic or tissue schizogony, the nucleus divides and the cytoplasmatic mass grows. The number of nuclear divisions and their intervals vary widely in different species. So does the ultimate size of the exoerythrocytic schizont which in some species may reach a diameter of 3 mm. After completion of the nuclear divisions, the cytoplasm segregates and merozoites are formed, consisting of a single nucleus and cytoplasm. In contrast to the erythrocytic stages, exoerythrocytic schizonts do not contain pigment. Conspicuous round vacuoles have been described in the exoerythrocytic schizonts of \textit{P. vivax}, while clefts, floculci, or inclusions have been reported in other plasmodia, which appear to be typical of the species and may permit its identification from the morphology of the exoerythrocytic schizont. \textit{P. malariae}, \textit{P. ovale}, and \textit{P. brasilianum} are the only primate plasmodia in which the host cell nucleus is enlarged. The number of merozoites produced by one exoerythrocytic schizont is estimated to be approximately 2000 in \textit{P. malariae}, 10 000 in \textit{P. vivax}, 15 000 in \textit{P. ovale}, and more than 30 000 in \textit{P. falciparum}, roughly corresponding to 12, 14, 15 and 16 nuclear divisions. The diameter of the mature exoerythrocytic schizont is approximately 45 \mu m in \textit{P. malariae} and \textit{P. vivax}, 70 \mu m in \textit{P. ovale}, and 60 \mu m in \textit{P. falciparum}. The minimum duration of the exoerythrocytic development of
primate plasmodia varies between six and 15 days. The site of primary exoerythrocytic schizogony in reptilian plasmodia is still unknown. For mammalian plasmodia, it is now generally accepted that the merozoites originating from cryptozoic schizonts, i.e., primary schizogony or exoerythrocytic schizogony arising directly from sporozoites, can only invade red cells, while those of avian plasmodia can only invade new tissue cells, giving rise to metacryptozoic schizonts, i.e., a second round of schizogony. After having passed through at least one subsequent tissue schizogony - phanerozoic schizogony - the avian phanerozoic merozoites can ultimately invade erythrocytes.

The erythrocytic merozoites are ovoid or elongated structures and species-specific in size (the long axis in *P. falciparum* is 0.7 µm; in *P. vivax* 1.2 µm; and in *P. ovale*, 1.8 µm). They possess an external membrane covered by a distinct surface coat of parasitic origin (Mason et al., 1977). The apical region contains the paired organelles (rhoptries) and a few micronemes which may be involved in the invasion of the erythrocyte. The nucleus and a mitochondria-like organelle are found in or near the posterior region. During invasion of the erythrocyte, the anterior end of the merozoite attaches to the erythrocyte membrane, which after thickening forms a junction with the plasma membrane of the merozoite (Aikawa et al., 1978). The erythrocyte invaginates to form a parasitophorous vacuole in which the parasite eventually lies; the junction between erythrocytic membrane and plasma membrane of the merozoite assumes the form of a ring which appears to travel along the merozoite surface. During this process of invasion the surface coat of the merozoite is lost and apparently remains outside the erythrocyte.

Once within the parasitophorous vacuole, the parasite transforms rapidly into a young trophozoite which is finally surrounded by two membranes; the inner one is the original parasite membrane and the outer one, which is contiguous with the exception of the cytosome area, is derived from the host erythrocyte. Mostly haemoglobin is ingested, probably only through the use of the cytosome, and it is digested in numerous phagosomes located in the parasite's cytoplasm to produce typical malarial pigment (haemozoin).

During this process the parasite grows; amoeboid movement, initially marked in younger trophozoites, decreases when the trophozoite approaches a full-grown stage, assuming a more-or-less rounded shape. The nuclear material increases and undergoes several divisions, usually three to five in mammalian species, resulting in the appropriate number of nuclei which are situated in a cytoplasmic syncytium until the parasite ultimately divides to form erythrocytic merozoites. The erythrocytic parasite containing many nuclei and syncytial cytoplasm is called a pre-segmenter or pre-schizont, and the parasite containing fully differentiated merozoites is called a (mature) schizont. The mature schizont bursts, liberating the individual merozoites which, in mammalian species, can only invade erythrocytes. In avian and reptile plasmodia they can also invade tissue cells and produce phanerozoic schizogony or, in the case of reptilian malaria, initiate paraerythrocytic schizogony, i.e., schizogony in blood cells other than erythrocytes.

During the process of blood schizogony the infected erythrocytes may retain their normal size as in *P. malariae* and *P. falciparum*, or become enlarged and round as in *P. vivax*, or enlarged and deformed as in *P. ovale*. Typical dots or clefts may develop in the stroma of the infected red blood cells. The choice of the human erythrocytic cell may be apparently universal, as in *P. falciparum*, or specific, as for example the Duffy-positive young erythrocytes in *P. vivax* (Miller, 1977). The duration of blood schizogony is generally 24 hours or a multiple of 24 hours, usually 48, or 72 hours, but there are notable exceptions, e.g. *P. gallinaceum* with a 36-hour asexual cycle. The mechanism of synchronicity observed in some species such as *P. knowlesi* and *P. chabaudi* is largely unknown.

Growth and division synchrony of *P. berghei* in mice can be augmented by specific photoperiodic rhythms. This mechanism and vascular sequestration of parasitized erythrocytes are apparently controlled by the pineal gland. Photoperiodically augmented synchrony is lost after pinealectomy; in the pinealectomized animal it may be restored by ubiquinone and members of the vitamin K group (Arnold et al., 1969 a,b,c).
Upon invading a new erythrocyte the merozoite can either initiate renewed blood schizogony or develop into a female or a male gametocyte (a macrogametocyte or a microgametocyte); these gametocytes are elongated in Laverania and Huffia but round in the other mammalian and avian subgenera. The early gametocyte stages have a more solid appearance, a smaller vacuole, and less amoeboid activity than the schizogonic trophozoites but exhibit a similar pattern of pigment granules. The mature macrogametocytes usually show a compact nucleus and an accumulation of pigment near the nucleus. In microgametocytes the nucleus is larger and less compact, often with a "spongy" appearance. The gametocytes are still surrounded by the host erythrocyte's membrane. Usually, the number of microgametocytes is notably less than that of macrogametocytes. The exact duration of gametocytogony is not known. In P. vivax it is assumed to be four days, while in vitro studies suggest a minimum duration of eight days in P. falciparum. In a number of mammalian plasmodia, gametocytogony may be initiated directly from cryptozoic merozoites, and in avian malarias from phanerozoic merozoites. When taken up by a suitable arthropod, the gametocytes transform into gametes. Macrogametocytes shed the erythrocyte membrane and become mature macrogametes without any evident morphological change. In contrast, microgametocytes undergo a complete transformation: the nucleus divides three times, forming an average of eight new nuclei which combine with cytoplasm to form microgametes having a very specific organelar structure. The exflagellated microgametes then tear free and move actively toward the macrogametes and invade them; after entry, the cytoplasmic material of the macrogamete and microgamete combine to form the zygote. Exflagellation usually takes 10-15 minutes, and microgamete entry one minute (Sinden & Croll, 1975; Sinden et al., 1978); these processes are separated by the time required for the microgamete to reach the macrogamete and to align itself.

The zygote remains immobile for some time. After both nuclei combine, the zygote elongates to form an ookinete, the broad anterior region of which appears to be rather inflexible, containing a projecting, truncated papilla with an apical complex (Sinden, 1975). The ookinete is actively motile (Speer et al., 1975). It moves toward the arthropod host's intestinal epithelium, enters it, and comes to rest beneath the basal lamina, forming an oocyst. In mammalian plasmodia, penetration of the gut epithelium by the ookinete usually takes place in the anterior part of the midgut of anopheline mosquitoes. The oocyst grows, surrounded by a smooth wall (Strome & Beaudoin, 1974). The cytoplasm maintains its syncytial structure, while intensive nuclear division occurs at a speed which is largely dependent on the environmental temperature. The pigment, carried along by the macrogamete, still remains in the oocyst. Ultimately the cytoplasm divides to form sporozoites. By this time the oocyst may have reached a diameter of 500 \( \mu \text{m} \) or more. The sporozoites emerge from the oocyst into the haemolymph through small individual holes or through larger openings where the oocyst wall has torn away (Sinden, 1975). In mammalian and avian plasmodia, most sporozoites - elongated, fusiform, and highly motile structures - migrate to the salivary glands of the arthropod host. They penetrate the glandular cells (Sterling et al., 1973) and ultimately reach the lumina of the salivary ducts from which they are able to reach the vertebrate host with the next bite of the arthropod. Sporogony, i.e., the period from gametocyte maturation until the development of infective sporozoites in the salivary glands, takes between eight days and four weeks. The number of sporozoites produced by one oocyst varies according to the plasmodium species. In P. falciparum it is estimated to be 10,000 (Pringle, 1965), which under optimum environmental conditions indicates a 12-hour cycle of nuclear division in the oocyst. Gametocyte maturation and gamete, zygote, and ookinete formation may also take place in an a priori unsuitable host or in vitro, but further development is normally limited to the natural vector.

Mitotic and meiotic phases of nuclear division in plasmodia are still the object of controversy. Although some observers have described the presence of classic chromosomes, such claims have not been substantiated. This does not preclude mitotic division of nuclear material. The first division in the post-zygote stage is assumed to be meiotic, accompanied by the disappearance of the nuclear membrane (Garnham, 1966). This is apparently followed by mitosis. Howells & Davies (1971) reported electron microscopic studies suggesting mitotic division in the oocyst stages of P. berghei. There seems to be agreement that the nucleus of sporozoites, merozoites, and gametocytes is haploid and that division during exoerythrocytic and erythrocytic schizogony is mitotic, although not typically eukaryotic, since the nuclear membrane remains intact during division.
The past decade has brought a fundamental revision of the concept of exoerythrocytic schizogony in the subgenera *Plasmodium* and *Vinckeia*. Originally it was held that the species of these subgenera underwent secondary exoerythrocytic schizogony. This would explain the relapsing nature in some of these species. However, this hypothesis is now being questioned. The relapses in *P. cynomolgi*, *P. fieldi*, *P. ovale*, *P. simiovale* and *P. vivax* are now being associated with the presence of latent cryptozoites ("hypnozoites", Markus, 1978), the existence of which was shown by Landau (1973) in *Gnomomyx surdaster* and substantiated for *P. vivax* through the clinical observations of Shute et al. (1976) and Ungureanu et al. (1976).

In a recent experimental study by Krotoski et al. (1980) 12 million *P. cynomolgi bastianelli* sporozoites were inoculated intravenously into a rhesus monkey and biopsy specimens taken at intervals of 2, 12, 24 and 48 hours and 7, 50, and 102 days after infection. The biopsy specimens taken on day 7 contained a sufficient number of large schizonts of approximately 35 μm diameter as well as a few very small schizonts within the parenchymal cells. These schizonts were 2.9-5.5 μm in size and had a single nucleus with a small amount of bluish cytoplasm. The biopsy specimen taken on day 50 after inoculation contained schizonts that could be called "relapse bodies" of approximately 30 μm as well as small ones of 5.7-7.0 μm possibly hyperzoites. This finding should be taken as a demonstration of latent stages of the parasites.

1.2 In vivo cultivation

In the last five years major advances have been made in the field of in vitro cultivation of malaria parasites and these have markedly affected progress in the fields of parasite biology, chemotherapy and immunology. The erythrocytic stages of *P. falciparum* can now be continuously cultivated in vitro and the techniques are widely used in laboratories throughout the world. In addition, the growth of the exoerythrocytic stages has been obtained in vitro although this technique is not yet fully optimized.

1.2.1 Erythrocytic stages

(a) *P. falciparum*

Trager (1976) and Trager & Jensen (1976) first reported the continuous cultivation of *P. falciparum* from *Aotus* monkeys using a continuous flow system developed earlier by Trager. Soon after, a greatly simplified method - the Petri dish/candle jar technique (Jensen & Trager, 1977) - was developed which facilitated the examination of various parameters and allowed an improvement to be made in parasite growth and production. Subsequently this technique has been used to cultivate several strains isolated from human infections in different geographical localities by several laboratories including that of the Rockefeller Group (Jensen & Trager, 1978; Trager & Jensen, 1978). Using the candle jar technique permits increases in parasitaemias of sevenfold to eightfold/cycle, and peak parasitaemias of 15-20% can be obtained if the medium is replaced every eight to 12 hours.

The original methods of Trager and Jensen have been scaled up by themselves and others in order to increase the yield of parasites for immunological, biochemical and chemotherapeutic studies. Trager and his co-workers have improved the continuous flow technique (Trager, 1979) and developed a semi-automatic apparatus (Jensen et al., 1979), of which a similar system has also been developed by Chin (1979). These culture methods are already perfectly suitable for the convenient production of parasite material on a scale large enough for most laboratory purposes although they will require modification before they can be applied on an industrial scale.

A prerequisite for the industrial "scale-up" of the culture systems is a suitable replacement for human serum, which is both expensive and difficult to obtain in large quantities. To this purpose several types of sera and serum replacements are being tested and horse serum has recently been shown to maintain the parasite in continuous culture (Butcher, 1979).
Using present methods, the growth of the parasite in continuous culture is asynchronous (Trager & Jensen, 1976) but for most studies it is essential that synchronous cultures should be obtained. Although a physiological method for synchronization has yet to be found, mechanical methods which separate rings or mature forms from other blood stages have been developed by several laboratories (Pasvol et al., 1978; Reese et al., 1979) and these are being used in attempts to grow synchronous cultures of *P. falciparum*.

(b) Other malarial parasites

The Trager-Jensen system has been used for continuous cultivation of other malarial parasites. Two simian malarial parasites, *P. knowlesi* (Gao Min-xin et al., 1979) and *P. fragile* (Chin et al., 1979) have now been successfully cultivated, the latter being of importance since it provides a good alternative model for *P. falciparum*. In addition the continuous cultivation of *P. berghei* in cells from *Mastomys natalensis* has been achieved (Guru & Sen, 1980) and this may have applications to the cultivation of *P. vivax*.

(c) Gametes

The continuous cultivation techniques are already being used to study the process of gametocytogenesis in malaria parasites. Smalley (1976) had shown that very young gametocytes, as young rings, invade the peripheral blood along with asexual rings which originate from the same population of schizonts. The gametocytes in contrast to the asexual stages take around 10 days to develop. Although attempts to induce gametocytogenesis in culture have been made by several laboratories, it has only recently been demonstrated that viable gametocytes infective to mosquitoes can be obtained in vitro.

1.2.2 Exoerythrocytic stages

Although the exoerythrocytic stages of avian malaria parasites have been grown previously in vitro, it was not until recently that the successful growth of these stages of mammalian parasites was obtained. In 1979, Strome et al. described the development of late schizonts of *P. berghei* in embryonic rat brain and liver and embryonic monkey brain following inoculation of the cultures with sporozoites and Sinden & Smith (1980) have confirmed these results in rat glial cells. These studies are particularly important for the development of an in vitro system for the screening of tissue schizontocides and for the provision of material for immunological studies.

1.3 Parasite separation and isolation

Several methods have recently been developed for the isolation of specific parasite stages which are required for the characterization of antigens as well as for reliable biochemical studies and studies on the basic biology of the parasite such as parasite invasion. A variety of gradient and sedimentation methods have been used, e.g. plasmagel (Pasvol et al., 1978), physiogel (Reese et al., 1979), metrizamide gradients (Eugui & Allison, 1979), and ficoll gradients (Mrema et al., 1979). Although separation into the ring or schizont stage may be obtained by many of these methods, none is ideal and the degree of separation may depend on the strain or species of parasite studied. It has also been shown that exposure of a culture to 5% sorbitol will kill all the late stages of the parasites but leave the ring stages uninjured.

In addition to these methods, the technique of carrier free cell electrophoresis has been adapted for the separation of malaria parasites (Suzuki et al., 1979; Heidrich et al., 1979). This method permits the stage specific separation of infected cells from uninfected cells, erythrocytes of different ages and plasmodial merozoites. A second novel method for the stage specific separation, based on the DNA content of the parasite and using a fluorescence activated cell sorter following labelling of the parasite DNA with bis-benzemidine, has also been developed (Howard & Battye, 1979).
The successful isolation of viable invasive stages of the merozoite and sporozoite has been essential for recent studies on the development of stage specific vaccines and on parasite invasion. Culture systems have been used for the natural release of erythrocytic merozoites. Dennis et al. (1975) have developed short-term cultures of P. knowlesi in chambers, the floor being a polycarbonate sieve through which merozoites are drawn as they are released. More recently a simple method for the isolation of merozoites, based on the principle of attaching erythrocytes to sepharose beads through a ligand which does not attach to merozoites has been developed by the Institut Pasteur (David et al., 1978; David & Hommel, 1979). This method has now been adapted for the separation of P. falciparum merozoites (Billiault & Ambroise-Thomas, 1980).

Density gradient and filtration techniques have also been developed for the isolation of malarial sporozoites. Beaudoin et al. (1977) used biphasic discontinuous renografin gradients to recover sporozoites which retained their infectivity and immunogenicity, and more recently an improved procedure, using membrane screen filters, has been developed by the same group (Wood et al., 1979). This latter method significantly reduced the contaminated microorganisms but the gradient method was more effective in removing viable microorganisms.

1.4 Parasite invasion of host cells

The process of invasion of host cells by the parasite is potentially important for the control of the disease by interference by immunological or chemotherapeutic methods but a great deal has still to be learned of the mechanism before this approach can be exploited.

The merozoite is surrounded by a pellicle of three membranes and a row of subpellicular microtubules, and an apical complex comprising electron dense rhoptries and micronemes is found at the anterior end (Aikawa, 1977; Bannister et al., 1977). In P. lophurae, and possibly P. falciparum too, the rhoptries from erythrocytic merozoites contain a histidine-rich protein which may possibly be involved in the invasion process (Kilejian, 1976). A surface coat is present on the surface of both sporozoites and merozoites. The coat of merozoites is electron dense, fibrillar and appears to be attached to the inner pellicular membrane (Bannister et al., 1975; Mason et al., 1977). It appears also to consist of protein or glycoprotein since it is removed by trypsin and gives cytochemical reactions which are typical of acidic glycoproteins (Bannister et al., 1975). It contains strain specific antigens (Mason et al., 1977; Miller et al., 1978).

As a result of advances in parasite culture and merozoite preparation the process of invasion of the erythrocyte by the malarial merozoite has been well documented morphologically. Invasion consists of attachment of the anterior end of the parasite to the erythrocyte, deformation of the erythrocyte and entry of the parasite by erythrocyte membrane invagination (Dvorak et al., 1975). The area of the erythrocyte membrane to which the merozoite is attached becomes thickened and forms a junction with the plasma membrane of the merozoite. As the merozoite enters the invagination, the junction, which is in the form of a circumferential zone of attachment between the erythrocyte and merozoite, moves along the confronted membranes to maintain its position at the orifice of the invagination (Aikawa et al., 1978).

The mechanism by which the erythrocyte membrane is invaginated with the consequent formation of the parasitophorous vacuole is unknown. It is possible that it may result from the release of the histidine-rich protein of rhoptries (Kilejian, 1976). However, Bannister et al. (1977) have proposed that it may be initiated by the injection of lipid. The parasitophorous vacuole is not a simple invaginated erythrocyte membrane since it appears from histochemical localization studies with the electron microscope that its polarity is reversed (Langreth, 1977). Freeze fracture studies also have indicated that the structural organization of the parasitophorous membrane differs markedly from that of the erythrocyte membrane (McLaren et al., 1979).
Merozoites will only attack and invade erythrocytes from susceptible species. Experimental evidence for specific interactions between merozoite and erythrocyte surfaces rests on the relative susceptibility to invasion of various types of erythrocytes as well as blocking effects of enzyme treatments and specific antibodies (Miller et al., 1975, 1977). Nevertheless a well-defined receptor has not been identified. The Duffy blood group stands alone among erythrocyte components at the present time having been associated with the attachment of *P. knowlesi* and *P. vivax* to human erythrocytes (Miller et al., 1977).

1.5 Metabolism

The basic reason for studying the biochemistry of the parasite is to obtain a thorough understanding of the physiological mechanisms of the parasite and its relationship to the host cell, in order to develop rationally new methods of chemotherapeutic attack and of antigen production.

1.5.1 Plasmodial membrane biology

Studies on the membranes of malarial parasites only started in the mid-1970s, when techniques which had been recently developed in other systems were applied to the parasite in the infected cell.

A major effort is being made to isolate the various membrane components of infected cells for biochemical, immunological and biophysical studies. Markers specific to the various fractions are being sought, and the studies are being correlated with electron microscopy and freeze fracture, and cytochemical techniques. Changes during the life cycle of the parasites are also being studied and attempts are being made to relate these changes to the antigenicity of the parasite and the parasitized erythrocyte. These studies are particularly important for the isolation of stage-specific antigens for the development of vaccines and specific immunodiagnostic tests.

Electron microscope studies with *P. falciparum* have shown that infected erythrocytes develop knob-like protrusions as the parasite reaches maturity within the host cell (Langreth et al., 1978) and that immunogenic proteins of parasite origin are present on these knobs (Kilejian et al., 1977; Langreth & Reese, 1979). After long-term in vitro cultivation these knobs may be lost in certain strains and this phenomena is correlated with an inability to concentrate the mature forms by the use of gelatin sedimentation techniques (Langreth et al., 1979). Freeze fracture studies on *P. knowlesi*-infected erythrocytes have also indicated that there is a structural rearrangement of the erythrocyte membrane during growth of the parasite (McLaren et al., 1979). These alterations include a loss in the number of intramembranous particles on the membrane which may correlate with the permeability changes observed in the infected erythrocyte, such as those described by Neame & Homewood (1975), and Sherman (1977).

Biochemical studies on the membranes of the erythrocytic stages are being performed in several centres in the world. These studies have depended on the development of methods for the fractionation of infected cells. Several methods are currently available but they give variable yields with different degrees of contamination. A recently developed method using nitrogen cavitation gives reproducible results for the removal of erythrocyte membranes from infected cells. This method may also be capable of the sequential removal of the parasitophorous vacuole and parasite membrane but this has to be confirmed (Wallach & Conley, 1977). Considerable progress in this field has been made in the last few years. In *P. knowlesi* (Wallach & Conley, 1977; Schmidt-Ullrich & Wallach, 1978), *P. berghei* (Yuthavong et al., 1979), and *P. chabaudi* (König & Mirtsch, 1977; Yuthavong et al., 1979) infections, new proteins have been found in the erythrocyte plasma membranes of schizont infected cells but not in uninfected cells. This has led to the hypothesis that new proteins are inserted into the erythrocyte membrane. These studies have been extended in *P. knowlesi* where two species-specific antigens, which induce antibody production in immune hosts, have been observed on the surface of schizont infected monkey erythrocytes (Schmidt-Ullrich et al., 1979a,b). Similar studies (Deans et al., 1979) have indicated two stage specific antigens on the surface of infected cells and of merozoites which may be species specific.
These studies have also indicated that glycoprotein components are lost from the erythrocyte membrane during parasite growth (Wallach & Conley, 1977; Trigg et al., 1977; König & Mirtsch, 1977; Schmidt-Ullrich & Wallach, 1978; Shakespeare et al., 1979b) and alterations occur in the spectrin components and in the phosphorylation of these structural proteins (Wallach & Conley, 1977; König & Mirtsch, 1977; Chaimanee & Yuthavong, 1979). These changes may be correlated with the changes in shape and permeability of the erythrocyte during infection.

Alterations in the activities of some membrane-associated enzymes have been detected in plasmodia infected cells but the significance of these changes is not yet known (König & Mirtsch, 1977).

There are many differences between the lipid composition of malarial parasites and that of the host erythrocyte and these studies indicate that lipid analyses may possibly be used as an indication of the purity of parasite preparations and also may indicate some areas for chemotherapeutic attack (Holz, 1977; Holz et al., 1977).

Studies have also been made on the surface structure of sporozoites since morphological studies had shown that protective antisorpzoite antibodies react with the surface of that parasitic stage (Nardin & Nussenzweig, 1978). A protein with a molecular weight of 41,000 has been detected by immunoprecipitation techniques as the primary antigen on P. berghei which reacts with specific antisera. The molecular weight of this main surface antigen of sporozoites of P. knowlesi is different from that of P. berghei (Gwadz et al., 1979). Protective antibodies against this protein have now been produced by hybridoma techniques (Yoshida et al., 1980).

1.5.2 Other metabolic pathways

(a) Carbohydrate metabolism

Recent work on carbohydrate metabolism of erythrocytic stages of P. knowlesi has indicated that the catabolism of glucose through the cell cycle varies with an increase in glycolysis during growth from the ring to the late schizont and subsequent further oxidation of pyruvate at the schizont stage (Shakespeare et al., 1979a). In addition, study of the respiratory requirements of P. falciparum in vitro has indicated that the parasite is an obligate microaerophile (Scheibel et al., 1979).

(b) Protein synthesis

Cell-free protein synthesizing systems have been developed for malaria parasites, particularly as an alternative method for antigen production. Sherman (1976) and Sherman & Jones (1976) used ribosomes from P. knowlesi and P. lophurae respectively with a rabbit reticulocyte extract and more recently Eggitt et al. (1979) utilized P. knowlesi RNA in their systems. The proteins synthesized in those systems correlate with those shown to be synthesized in vitro (McColm et al., 1976).

(c) Co-factors

Relatively few co-factors seem essential to the intracellular development of the malarial parasite. These are para-aminobenzoic acid which is presumably required for the synthesis of folates, biotin, and pantothenate (Trager & Brohn, 1975). Malaria parasites differ from their hosts in that they synthesize folate co-factors de novo and consequently this pathway has been the site of chemotherapeutic attack by the action of sulfon drugs in association with pyrimethamine or another related antifolate. It has been assumed that the action of these drugs is on nucleic acid metabolism, but the report by Smith et al. (1977) indicating that N5-methyltetrahydrofolate may be utilized for methionine biosynthesis might suggest that the role of folate in the malaria parasite is not solely a precursor for the thymidylate pathway. These studies have application to studies on the mode of action of antifolate drugs.
(d) **Nucleic acid synthesis**

The intraerythrocytic stages of plasmodia are capable of synthesizing purine nucleotides and apparently deoxycytidylate by salvage synthesis. Data obtained by studying the incorporation of radioactive precursor molecules into intact cells and kinetic experiments on purified enzyme preparations suggest biosynthetic routes which, generally, are similar to those of the host's cell metabolism. However, details on the regulation of both the uptake of nucleotides and bases into the intraerythrocytic stages of plasmodia and of the metabolic routes involved in this incorporation are still lacking (Königk, 1977; Van Dyke et al., 1977).

1.6 **Characterization of plasmodial strains**

It is fundamental to any control programme that the precise identity of the parasite can be established. This is particularly true in relation to the development of vaccines and to epidemiological problems such as the understanding of the basis for a spread of drug resistance.

Characteristics used to differentiate strains have included morphological features, relapse patterns, drug response, infectivity to various vector species and immunological differences. More recently biochemical techniques have been used to examine genetic differences between isolates of both rodent and human malarial species. Two methods have been principally used so far: (1) enzyme electrophoresis which reveals differences in the products of individual gene loci, and (2) DNA characteristics which reveal differences in the total gene information of the organisms. Enzyme electrophoresis has revealed considerable genetic diversity in populations of malarial parasites and has enabled populations reproductively isolated from one another to be distinguished by their pattern of enzyme forms (Carter & Walliker, 1977; Carter, 1978). In each subspecies in which enzyme polymorphism occurs, the frequencies of each combination of enzyme forms suggest an extensive degree of random mating in the population. Populations of *P. falciparum* are also enzymatically polymorphic, similar enzyme variants being found in isolates from several parts of the world (Carter & Voller, 1975; Carter & Walliker, 1977). Studies are currently proceeding to develop more sensitive techniques for strain characterization and to adapt these techniques for studies with *P. falciparum*.

1.7 **Genetics of drug resistance**

Studies on the genetics of drug resistance are essential to determine how drug-resistant strains arise, how these may give rise to more resistant forms as a result of genetic recombination or spontaneous mutation, followed by selection, and how resistance spreads in the field. This is of practical importance to control programmes. The studies have benefited from the developments in strain characterization described above since isoenzyme forms represent products of a single genetic locus and have provided extra markers to be studied in crosses between resistant and sensitive strains.

The results show that resistance to both chloroquine and pyrimethamine are inhibited according to normal Mendelian rules (Beale et al., 1978). As far as can be decided with present techniques, rodent malarial parasites at least show a regular alternation of diploid and haploid phases. The blood forms are haploid but it is not at present possible to state from genetical data the precise stage in the life cycle when meiosis takes place (Walliker et al., 1975). The demonstration that resistance to pyrimethamine and chloroquine is due to the occurrence of gene mutations, which once arisen may spread through a population, is important in relation to the problem of drug resistance in man (Rosario et al., 1978). Of particular interest in these studies is the rather surprising finding that, even in the absence of drug pressure, chloroquine-resistant parasites appear to have an advantage over chloroquine-sensitive forms, at least in the *P. berghei/mouse* model. A similar process in human malaria might be expected to produce a rapid dissemination of chloroquine-resistant parasites.
This work is of particular importance in view of the recent demonstration that stable chloroquine resistance can be induced under laboratory conditions in 15 generations in an originally chloroquine-sensitive strain of *P. falciparum* from West Africa - an area where chloroquine resistance has yet to be found (Nguyen-Dinh & Trager, 1979).

1.8 Mode of action of drugs and mechanisms of drug resistance

Studies on parasite biochemistry related to the mode of action of blood schizontocides are being carried out in several laboratories but, to date the complete mode of action of any antimalarial drug is unknown. Priority is given to work on chloroquine and at present most of the work is conducted on the *P. berghei* model.

It has been known for several years that malaria infected erythrocytes concentrate blood schizontocides (4-aminoquinolines, mepacrine, quinoline methanols such as mefloquine and phenanthrene methanols) by a saturable energy-dependent process involving high affinity binding sites and that in the case of chloroquine at least, resistant strains have a deficiency in this concentration mechanism (Fitch et al., 1975a,b). Further work by Fitch's group has indicated that erythrocyte surface components determine the affinity with which chloroquine is accumulated and thereby determine whether or not the malaria parasite will be susceptible to the drug (Fitch et al., 1978). Recent studies suggest that an aggregated form of ferrisprotoporphyrin IX has the characteristics of the chloroquine receptor of erythrocytes infected with chloroquine-susceptible *P. berghei* (Chou & Fitch, 1979). Ferrisprotoporphyrin IX exists in the parasite only transiently in a form accessible to the drug and it is suggested that the binding of chloroquine delays the processing of this transient form into malaria pigment (Chou & Fitch, 1980).

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2. THE VECTOR

The residual insecticides and their straight-forward impact on malaria transmission served to push to the background studies on vector biology and behaviour. However, it was later realized that knowledge of the vector is essential for understanding the reasons for the failure of the insecticides and devising remedial measures. Moreover, vector resistance, rising cost of insecticides and the problem of environmental pollution stimulated exploration of non-chemical methods of vector control which demanded an even more intimate knowledge of the vector. There has thus been a renewed interest in the study of vector biology, genetics, etc., with where relevant, emphasis on quantitative rather than on qualitative studies.

2.1 Vector biology

2.1.1 Vector resting and feeding habits

Outdoor resting and feeding may result in insufficient or no contact between the vector and indoor residual deposits. There has usually been inadequate pre-spray information to determine whether exophily and exophagy were inherent or induced. Anopheles farauti in British Solomon Islands showed lesser entry into houses and fed earlier in the night thereby increasing the possibility of outdoor transmission. A similar shift in biting activity under insecticidal pressure was reported in An. balabacensis and An. minimus in Thailand. Exophily and exophagy or tendency to avoid treated surfaces was observed in the above two species in Thailand, in An. balabacensis in Bangladesh and in An. philippinensis in India. Forest clearance led to the disappearance of An. balabacensis which was however replaced by An. minimus.

An. culicifacies density in Pattukottai in India was reputed to have declined considerably as a result of changes in environment due to urbanization, developmental programmes and insecticide use. An increasing importance of the species with changing ecological patterns, on the other hand, was observed in Delhi following the engulfment of the riverine belt and other irrigated areas within the growing city. At the same time, the expansion of the piped water supply restricted An. stephensi (previously the sole vector) breeding to water storage tanks in construction complexes where it assumed a focal importance only. Although essentially endophilic, a small proportion of An. stephensi were found to rest outdoors in South Iran and in South India. Exophily was also recorded in An. dthali in Iran.

The replacement of an established vector (An. pulcherrimus) by another (An. hyrcanus) was recorded in Afghanistan in the wake of agricultural development. DDT spraying alone was found insufficient to interrupt transmission by the exophilic, exophagic and DDT-resistant An. hyrcanus and the partially exophilic An. pulcherrimus with a tendency to avoid treated surfaces. The latter authors advocated the introduction of fish as the chief measure of control in the rice growing areas.

An. darlingi in Surinam was found biting in the daytime although nocturnal activity predominated and An. nuneztovari was suspected to be a possible secondary vector although the X-chromosome of the local population resembled that of a Brazilian non-vector strain. An. darlingi fed during the day (mainly 50% biting outdoors) also in French Guyana and the vector/man contact occurred mostly on the verandah and outdoors. An. darlingi was reported in Brazil avoiding DDT-sprayed surfaces even though feeding indoors. The uncontrollable character of human malaria in Western Venezuela was attributed to exophily of An. nuneztovari.
In the Congo in Africa, An. nili (generally regarded as a secondary vector) showed high man-biting activity indoors as well as outdoors causing a high level of transmission of malaria. An exophilic and exophagic member of the An. funestus subgroup (An. aruni) was reported possibly responsible for outdoor transmission in Transvaal. White remarked that while the endophilic An. gambiae s.s. is easily controlled by house-spraying, the inherent resilience of An. arabiensis could be an insurmountable public health problem. Pre-spray observations in Kisumu, Kenya showed An. gambiae to be the dominant species and more endophilic than An. arabiensis although the two species did not differ in infectivity or host preferences. Fenitrothion spraying reduced the overall density of both species but resulted in higher exophily and relative proportion of An. arabiensis. Highton et al. found a much lower sporozoite rate in An. arabiensis than in An. gambiae s.s. The former species was more exophilic and zoophilic and also the dominant species in the plains. A study lasting 18 months in Ethiopia showed An. gambiae s.l. and An. funestus to be partially exophilic while others such as An. pharoensis and An. nili were strongly exophilic. Vinogradskaya and Detinova found a correlation between the value of the spiracular index and the humidity in the environment and regarded on that basis An. funestus to be tolerant of dry conditions, An. gambiae as intermediate and An. pharoensis as a hygrophilous species. The governing influence of humidity on resting habits found supportive evidence in that both An. gambiae s.s. and An. arabiensis with chromosomal rearrangements favouring a drier environment rested indoors (with a higher saturation deficit during the night than outdoors) while those with arrangements adapted to relatively more humid conditions rested outdoors. The influence of humidity on resting probably explains the restless behaviour and high turn-over among the indoor resting An. gambiae s.l. and An. funestus observed in an unsprayed village in Nigeria.

In a study in Gambia involving women and children of different ABO blood groups, the former received on the average over seven times as many An. gambiae s.l. bites as the latter. Another study showed that the number of An. gambiae s.s. bites on infants, children, adolescents and adults had a proportion of 1:2:2.5:3 and thus age-related biting pattern is important for mathematical models on total transmission in a locality.

Precipitin analysis of Anopheleline blood-meal samples have yielded valuable information on host preferences. Data on An. funestus confirm the highly endophilic and anthrophilic habits of the species. An. gambiae s.l. in Ethiopia showed a declining man-fed proportion from year to year indoors and outdoors in sprayed areas. In Jirima in Nigeria, where the sampling registered a big advance, the mean human blood index was found to be 0.92 in An. gambiae s.s., and 0.50 in An. arabiensis. In Bendel State in Nigeria where few animals were present at the time of sampling, both species had fed almost exclusively on man. A sample of 88 smears of An. gambiae s.l. collected in outlet traps in DDT-sprayed dwellings in Togo were all positive for man. Results from Kenya showed in general a higher man-fed proportion of An. gambiae s.l. than An. arabiensis. An. nili showed a high anthropophilic index in Congo and Togo. Limited data indicated wide geographical variations in the degree of contact with man by An. maculipennis and An. sergenti. The year by year recovery of the proportion of An. maculipennis s.l. fed on man in houses formally sprayed with DDT in Yugoslavia may be due to the gradual loss of the insecticidal and irritant effect. An. labranchiae in Morocco and An. aconitus in Indonesia showed an impact of spraying in reduced man-fed ratio in treated houses and an increase in unsprayed outdoor biotopes.
2.1.2 Other studies

Reisen and collaborators conducted a number of studies in Pakistan on resting, feeding, swarming, migration, etc. of An. culicifacies and An. stephensi.\(^3\)\(^7\)\(^8\)\(^9\)

An. aconitus in Indonesia was found mostly resting outdoors and the endophilic fraction rested only on the lower levels of the walls\(^4\) so that effective control could be achieved by selective spraying of the lower portions of the walls only. Spencer\(^4\) in Papua New Guinea proposed a new method of age-grading An. farauti populations through examination of the intact ovaries. A phyto-ecological map was produced for a small island off the west coast of Korea to serve as an indicator of mosquito breeding places.\(^4\) A circular six meter high fence barrier had no effect on the movement of An. gambiae s.l. and An. funestus which apparently freely flew over the obstruction.\(^4\)

The spatial distribution pattern of An. sinensis larval populations in rice fields in Japan showed that the basic component was a single individual distributed contagiously over the field.\(^4\) Service\(^4\) constructed time-specific life-tables for An. arabiensis in rice fields and temporary pools in Kenya and estimated a mortality of 93% from hatching to emergence.

Deterministic computer-based simulation models were developed using the average daily survival rates for adults and immature stages based on data collected in the field.\(^4\)

2.1.3 Species complexes

Intensive polytene chromosome studies in Nigeria helped to elucidate the behavioural divergences between An. gambiae s.s. and An. arabiensis which are the two most anthropophilic and efficient vector members of the complex.\(^4\) An. gambiae s.s. predominated in the forest and humid savanna while An. arabiensis is more prevalent in savanna and steppe. However, both are capable of colonizing contrasting environments as typical rainforest and sahel savanna due to plasticity conferred by a remarkably high degree of chromosomal inversion polymorphism. Distribution of the two species depended upon chromosomal arrangements favouring microclimatological conditions particularly humidity and could be influenced by man (dry-season breeding grounds through irrigation or deforestation and urbanization favouring isolated populations of An. arabiensis). The cytogenetic technique could however be applied only to fully grown larvae or females with Stage IV ovaries. The electrophoretic analysis based on gene enzyme systems\(^4\)\(^9\)\(^5\) is applicable to individual males and females. However, the members of the An. gambiae species complex exhibit only a low degree of electrophoretic allele divergence\(^4\)\(^8\) and the biochemical key established by Miles\(^5\)\(^1\) could not unambiguously distinguish between An. gambiae s.s. and An. arabiensis.

The first mosquito species to be ever named and described primarily from cytotaxonomic studies was An. beklemishevi belonging to the An. maculipennis complex in the Soviet Union.\(^5\)

2.1.4 Experimental studies

An. gambiae species A showed a highly significant preference to land and feed on a subject when thermogenic sweating was induced but were not apparently influenced differently by host variables such as MN blood group, skin temperature, humidity and colour and forearm hair density.\(^5\)\(^3\) Studies using
a twin-funnel testing-device showed that An. gambiae s.s. flew twice as long as An. arabiensis after irritation by DDT and that the "bite and run" individuals in a population might raise an endophagic and exophilic population under DDT residual spraying.\textsuperscript{54} Offsprings of wild-caught An. sacharovi from Turkey (reported to be prematurely escaping from DDT-sprayed houses) showed a high flight activity and capacity to penetrate escape ports and a tendency to rest on DDT uncontaminated surfaces.\textsuperscript{55} Alekseev and Suvorova\textsuperscript{56} showed that DDT-resistant An. sacharovi females on 15-30 minutes contact with DDT-treated surfaces fed two to three times more actively indicating a higher epidemiological danger from DDT-resistant individuals where DDT is in continued use. A study involving 19 flight variables indicated that uninfected An. stephensi exhibited superior flight characteristics.\textsuperscript{57} The immediate departure outdoors after feeding by An. nuneztovari in Venezuela was apparently facilitated by its ability to evacuate immediately a relatively high proportion of the fluid ingested, high flight activity, ability to pass through small orifices and possibly a preference for the lower temperature and higher humidity outdoors.\textsuperscript{58}

2.2 Vector genetics

Highly productive colonies and early separation of the sexes are essential prerequisites for sterile male release operations. An 80\% increase in the production of An. albimanus pupae was attained by controlling more accurately the water temperature and the number of eggs introduced into each rearing tray.\textsuperscript{59} A genetic sexing system was developed allowing the preferential elimination of An. albimanus females (during any of the four life stages) based on a recessive propoxur susceptible conditional lethal.\textsuperscript{60} A deterministic simulation model helped to establish the potential value of releasing male-linked translocation heterozygotes to control An. albimanus.\textsuperscript{61} A morphological mutant marker (stripe) visible in An. albimanus larvae and pupae was associated with chromosome 3 by the use of two Y-autosome translocations. Three distinct variants (based on pupal morphology) established from an An. albimanus population in El Salvador were found to differ significantly in their susceptibility to P. vivax and P. falciparum.\textsuperscript{62}

A laboratory strain of An. stephensi in India was found to be highly polymorphic with 32 various types of chromosomal rearrangements.\textsuperscript{63} A recessive autosomal mutant (colourless eye) in the larvae, pupae and adult was discovered in a laboratory colony of the species.\textsuperscript{64} An autosomal recessive colour mutant (green larvae) was isolated from another colony in India.\textsuperscript{65} Chemosterilized males of An. stephensi through irradiation of the male pupae proved permanently sterile and equal to untreated males in mating competitiveness and ability and survival.\textsuperscript{66} An. culicifacies was colonized in a small cage (30 cu.cm.) in Pakistan and polytene chromosome maps prepared.\textsuperscript{67} A sex-linked recessive mutant (rose eye) was found in the species.\textsuperscript{70} Baker et al\textsuperscript{71} studied cytologically the translocations and inversions induced by irradiation. Another study showed linkage groups and chromosomal relationships.\textsuperscript{72} Kanda and Oguma\textsuperscript{73} in Japan, showed through crossing experiments and study of salivary gland chromosomes that An. sinensis is reproductively isolated from An. sineroides and is also distinct from An. lesterii. Fraccardo et al\textsuperscript{75} showed that interspecific mating behavioural polymorphism exhibited by the eurygamous An. labranchiae and the stenogamous An. atroparvus is controlled by one or more genes located on the Y-chromosome.
Considerable genetic studies have been carried out by the Ross Institute of Tropical Hygiene, London, apart from the routine identification of members of the *An. gambiae* complex and work on insecticide resistance. An. *culicifacies* populations from Sri Lanka and Pakistan were found to differ in their polytene X-chromosomes. Members of the *An. gambiae* complex showed a strong tendency towards positive assortative mating (females mating with males of the same species in preference to males of another species). Such a tendency was less noticeable between strains of the same species. Genetic sexing mechanism was evolved by developing a strain each of *An. gambiae* s.s. and *An. arabiensis* with a gene for dieldrin resistance linked to the Y-chromosome. *An. gambiae* s.s. strains refractory to infection with *P. berghei berghei* and *P. yoelii nigeriensis* were developed and field studies conducted to test their refractoriness of *P. falciparum*. Using the white-eye linked marker a stock was produced with its X and Y-chromosomes from *An. gambiae* s.s. and its autosomes partly from the same species and partly from *An. arabiensis*.

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2.3 Resistance to insecticides (mechanisms and developments)

Present status

Physiological resistance of anophelines to insecticides is considered as one of the main obstacles to malaria control/eradication. Until 1975 when the last Expert Committee on Resistance of Vectors and Reservoirs to Pesticides1 met, a total of 42 anopheline species had been reported to be resistant, of which 41 species were resistant to dieldrin, 24 to DDT and 21 of the latter to both DDT and dieldrin. Appearance of resistance to organophosphorus compounds and carbamates was also recorded in Anopheles albimanus in Central America and to organophosphorus compounds in An. culicifacies in India, An. sacharovi, An. hycrurus in Turkey, An. messeae in Romania, and An. sinensis in the Ryuka Islands. New records from other territories than those reported above have been made since then and up to the date of writing this report 11 species and 27 territorial records have been added. A total of 37 species have developed resistance to DDT, 46 to DDT/dieldrin and 31 of these to both the groups of insecticides. Thirteen species have been recorded showing resistance to OP compounds and five to carbamates as well.

Notable new records which may have contributed to the deterioration of the situation of malaria control are as follows.

The development of resistance to malathion by field populations of An. stephensi in Iran, Iraq and Pakistan, An. arabiensis in Sudan, An. culicifacies in India. The dramatic resurgence of malaria in Turkey and Syria during 1976-1977 may have been due to the multi-resistance of An. sacharovi to OP, carbamate and chlorinated hydrocarbon insecticides. Resistance has also spread to DDT and dieldrin in An. annularis in Pakistan, An. maculatus in Malaysia and is strongly suspected in An. minimus in N. Thailand. An. balabacensis (An. dirus) is also suspected strongly to have developed resistance to DDT in Thailand. New reports of resistance to DDT, dieldrin/HCH and malathion in An. sinensis have come from China and to DDT from Viet Nam. An. vagus from Viet Nam has also shown resistance to DDT and dieldrin/HCH and An. subpictus from the same country to DDT.

Multiple resistance in the vectors of malaria particularly in areas where intense use of pesticides is made in agriculture has been of great concern in recent years. Several species of mosquitoes are often found breeding in agricultural areas and are liable to be exposed to the insecticides employed in crop spraying. This spraying may have a suppressive effect on the mosquito populations for some time, especially when the treatments affect the adult flying population. However, this also results in suppression of the natural enemies of mosquitoes and exerts a high selection pressure for accelerated development of mosquito resistance to insecticides. Examples of such occurrences have been cited for An. stephensi multiple resistance in Iran and Iraq where a variety of insecticides are used in date-growing areas;

An. hyrcanus and An. sacharovi in the Chukurova plains of Turkey; An. arabiensis from Sudan; An. albimanus in El Salvador. Of these the most serious case studied so far is that of An. albimanus in El Salvador, where during August to December intense suppression of mosquito populations occurs due to numerous agricultural spray operations and each selection has been shown to coincide with changes in resistance. Changes in resistance have also been shown to be correlated with intensity of agricultural operations in different areas and occur to insecticides used most widely.

The recent multiple resistance records in An. sinensis in China have also been reported to be related to the agricultural use of pesticides.

Cross-resistance characteristics of malaria vectors under field/laboratory conditions

Whenever a vector develops resistance towards the insecticide used for its control (or considered for its control, should a control programme be developed such as in the African Region), it is usual to investigate the cross-resistance pattern under the field conditions as has been done for An. albimanus in Central America and recently for An. sacharovi in Turkey. This approach has not always been rewarding as cross-resistance patterns might be difficult to determine when the genes imparting resistance have a low frequency. Under such circumstances it may be advantageous to colonize the resistant strains of the vector concerned and submit these through generations to the selective pressure of various insecticides for determining their cross-resistance pattern. This has been done in particular with An. albimanus for Central America, An. culicifacies from India, An. stephensi from Iran and An. arabiensis from Sudan. The findings of these field and laboratory investigations can be summarised as follows.

An. albimanus in Central America

A strain of laboratory selected carbamate resistant An. albimanus was not resistant to pyrethroids bioresemethrin, bioallethrin and CRS 11451 (2-chloro-4, 5 methylene dioxyphenyl methyl 2,2,33-tetramethyl cyclopropane carboxylate), although resistance to cismethrin was 2.3 fold. Resistance to other carbamates of similar structure was very high (>100x). Carbaryl exhibited 74.8x cross-resistance followed by carbonolate (20.6x). Certain other carbamates exhibited the resistance ratio ranging from 2x to 7.5x.

Propoxur selected strain exhibited resistance to malathion, parathion and methyl parathion (>100x), chlorpyrifos methyl (6.5x), chlorphoxim (6.1x), chlorpyrifos (20.6x), fenitrothion (38x), Diazinon (21.4x).

A propoxur and ethyl parathion selected strain of An. albimanus was resistant to parathion but remained susceptible to fenthion. Strains of An. albimanus collected from the field were resistant to propoxur, malathion, fenitrothion, DDT and dieldrin.

An. arabiensis in Northern Sudan

A strain of An. arabiensis collected from Geziera area of Northern Sudan shows high resistance to DDT, dieldrin and malathion, but cross resistance does not extend to fenitrothion, propoxur and fenthion.
An. culicifacies in Gujrat, India

An. culicifacies in Gujrat, India has been shown to possess high resistance to DDT, dieldrin and malathion. In the laboratory it has been shown that the malathion resistance exhibits cross-resistance to fenitrothion, pirimiphos methyl, and to a lower level to chlorphoxim, phoxim and jodfenphos.

An. sacharovi in S.W. Turkey

High levels of resistance to both DDT and dieldrin had already been recorded before 1971. Resistance to fenitrothion has been shown and cross-resistance characteristics show either a low level of resistance to a number of OP, carbamates and pyrethroids or a low proportion of specimens resistant to these chemicals as well as an almost normal level of susceptibility to malathion, pirimiphos methyl, phoxim and chlorphoxim.

An. stephensi in Southern Iran

An. stephensi from Southern Iran had developed resistance to DDT and dieldrin and since 1964 malathion was substituted. Studies on cross-resistance showed a high level of resistance to DDT, dieldrin and malathion and potential for a moderate level of resistance to fenitrothion. Fenitrothion and malathion resistance mechanism appears to be distinct. High level of cross-resistance was noted for pirimiphos methyl and a medium level (4x - 6x) of resistance to chlorphoxim and jodfenphos and a very low level of resistance to phoxim.

Resistance mechanisms

The three main resistance mechanisms in insects known to date are altered site of action, increased metabolism and reduced penetration of the insecticide.

Examples of altered sites of action consist of insensitivity of the target enzyme acetyl cholinesterase to inhibition by the toxicant as observed in OP and carbamate resistant An. albimanus. Another example of a similar category is the mechanism due to a recessive gene Kdr, which imparts resistance to DDT and its analogues as well as pyrethroids.

Increased metabolism due to the enzyme DDT dehydrochlorinase which converts DDT to the relatively non-toxic DDE also is a major mechanism of resistance. Increased degradation of OP insecticides has been shown to be brought about by phosphatases, carboxylesterases, glutathione dependent transferases and mixed function oxidases. Research work at the Ross Institute on cross-resistance characteristics of An. stephensi selected with malathion showed susceptibility to fenitrothion and 8x resistance to malathion indicating a mechanism specific to malathion and other compounds having a carboxyl-ester bond. However, when fenitrothion was used for selection there appeared to be a simultaneous increase in tolerance to the other organophosphates including malathion. This indicates a common mechanism which increases tolerance to these insecticides and is independent of the malathion specific mechanism mentioned above.

Reduced penetration of insecticides has also been given as a reason for resistance, sometimes in combination with other factors.
The dynamics of resistance development

In view of the complexity of resistance mechanisms giving rise to a variety of cross-resistance patterns, it is difficult to forecast resistance development and its characteristics when control operations are planned. In the early stages the many genes which could result in the development of resistance are present at a very low frequency, therefore preliminary field tests or systematic laboratory selection may or may not provide any indication of the resistance pattern particularly when the investigations are carried out on a limited sample. One thus cannot be surprised that all studies carried out along these lines have failed.

The prospects of forecasting resistance development and characteristics are better when the vector population under attack has already been selected for resistance by massive applications of insecticides (especially their agricultural use affecting both larvae and adults of the vector). The selection pressure is then intense and the genes for resistance, if present, will constitute an advantage; their frequency will increase and might become high enough to be detected through routine surveys and laboratory selections. It is under such circumstances that Anopheles gambiae s.l. resistance to dieldrin was detected in a number of African countries where this insecticide was not used for public health purposes.

The WHO Expert Committee on Resistance of Vectors and Reservoirs of Disease to Pesticides (TRS 585, 1976) has discussed the dynamics of resistance on laboratory versus field populations. While much work has been done on selection in laboratory populations, the predictions from such laboratory selection may be rather limited because of differences in genetic potential and several important ecological factors. However, if laboratory selection leads to the development of resistance (positive results), it can be concluded that such a resistance is likely to occur in the field. Such selection experiments may give important information on the qualitative aspects of resistance such as the gene pool, frequency of distribution of resistant genes within a population. The Committee, therefore, strongly recommended that resistance in field populations of important vector species should be characterized in the laboratory. The information would indicate the usefulness of the insecticide in use and which will be the most suitable replacement insecticide. Two of the WHO collaborating laboratories, one in the UK and the other in the USA are carrying out such studies on important vector species such as An. albimanus from Central America, An. sacherovi from Turkey, An. culicifacies from India, An. stephensi from Iran and An. arabiensis from Sudan.

Prevention of resistance development

One of the obvious consequences of this situation is that, with the exception of a complete interdiction of any insecticide use (or even pesticides as some fungicides are chemically related to certain public health insecticides) there is no reliable means to prevent resistance development. To a large extent the dynamics of resistance development depends more on the variety of chemical groups and total quantity of insecticides applied in the study area, than from the application of a given insecticide, especially when organophosphates and carbamate resistance are considered. Banning all cyclodiene insecticides would have most probably prevented, or considerably slowed down, dieldrin resistance. Banning DDT and all DDT-analogues might have given the same results related to DDT resistance. There is unfortunately no guarantee whatsoever that banning malathion from agricultural use could slow down the development of malathion resistance if other OP or carbamate insecticides are massively used in agriculture; the list of chemicals whose application should be banned to prevent malathion resistance development would be so large that no agricultural country could afford to do it.
Insofar as insecticides are providing satisfactory control these could be used in vector control programmes and for crop protection. Could, however, measures be taken to delay the development of resistance? Various factors which are known or presumed to influence the kinetics of resistance have been grouped under three main headings, A. Genetic, B. Biological, and C. Operational. Factors in the first two categories are beyond operational control; however, knowledge of their contribution serves in assessing the 'resistance risk', i.e. the propensity for resistance inherent in a population. Factors in category C are under the control of the operator and could be given greater or lesser emphasis depending on the assessment of the resistance risk based on categories A and B.

It has become increasingly evident that the greatest opportunity for countering the evolution of resistance depends on our ability to limit the degree of selection pressure (according to the resistance propensity characteristics of the target population). Resistance may be expected to evolve more rapidly when the following conditions prevail:

1. a residual insecticide is applied which is closely related to an earlier used chemical;
2. the compound has prolonged environmental persistence where mosquitoes breed or rest;
3. applications are made at a low population density;
4. the treatment reaches and selects a high percentage of the population;
5. selection is directed against larvae, or worse still against both larvae and adults;
6. a thorough application is made, a geographically large area is covered and selection is applied against every generation of the population; and
7. the insecticide deposit is applied at, or deteriorates to, the level at which heterozygotes for the resistance factor will survive.

The slow response to selection occurs when:

(a) the population is diluted by immigration;
(b) population density is drastically suppressed by severe selection; and
(c) susceptible individuals have a reproductive advantage over the resistant counterparts.

The consensus emerging from accumulated knowledge on resistance is that if heterozygote-resistant individuals can be killed, resistance can be delayed or avoided for example, by preventing the decay of insecticidal deposit on sprayed surfaces. Thus research on formulations to maximize the lethal effect on heterozygotes would be most desirable.
Development of insecticides against which known resistance mechanisms would not be effective

Development of compounds to which the resistance mechanism is not effective is a common approach to the problem. A well-known example is the derivatives of DDT, synthesized to avoid the action of DDT-ase, e.g. deutero-DDT and O-chloro-DDT which cannot be attacked by the DDT-ases. Some field trials were carried out using these chemicals for control of malaria transmitted by DDT-resistant anophelines but there appeared to be cross-tolerance to these chemicals as well.

Early detection of resistance

Early detection of resistance by use of discriminating concentrations of insecticides has been advocated by the 22nd WHO Expert Committee on Insecticides. (WHO, TRS, 585, 1976).

Dieldrin-HCH

The guidelines in this respect (0.4% for 1 hour - adults and 0.1 ppm for larvae) are simple and clear and the impregnated papers are stable. The tests are little temperature dependent. The practical use of this may not be much, however, because there are very few countries or areas where cyclodieneas are used anymore (although Sri Lanka might be one). Thus with very few exceptions the discriminating concentrations can give the exact constitution of the populations with regard to the susceptibility or resistance.

DDT

The guidelines of the Expert Committee on this are also simple (4% for 1 hour - adult and 2.5 ppm - larvae) and the impregnated papers are stable and the tests are little temperature dependent. Discrimination between susceptible and adult anophelines is by no means as clear-cut since the magnitude of resistance is not always very great. Interpretation of the field data is not always easy and between 2% and 20% survival verification is required which can be carried out by using a two-hour exposure. Variability in the base-line data for various species has been noted for DDT.

OP, carbamates, pyrethroids and IGRs

Tentative discriminating concentrations of malathion (5% for 1 hour - adult, and 3.125 ppm - larvae) fenitrothion (1% for 2 hours - adult, and 0.125 ppm - larvae), fenthion (2.5% for 1 hour - adult, and 0.05 ppm - larvae), propoxur (0.1% for 1 hour - adult) with some exceptions have been suggested. The test papers are not very stable and highly temperature dependent. The same situation exists for pyrethroids. Very little is known about the Insect Growth Regulators and the Insect Development Inhibitors. Test methods based on the inhibition of emergence have been suggested.

The above presentation has highlighted the extreme complexity of the insecticide resistance phenomenon in general and provided more detailed information regarding some major malaria vectors. It is essential to continue and even develop further field and laboratory investigations related to insecticide resistance dynamics, cross-resistance spectrum and biological mechanisms involved. At the same time one may draw some conclusions of practical importance for a better monitoring of resistance development in the field, as well as regarding the practical potential offered to malaria control by the few alternative insecticides which could be considered for house spraying.
Resistance monitoring

The routine monitoring of resistance is based on the use of critical concentrations for given exposure periods. These were established on a worldwide basis and do not always fit closely enough with the susceptibility characteristics of the local vector. Some vectors are less susceptible than the average and have already been granted special "critical concentration x exposure periods", such as the members of the An. maculipennis complex. It would be appropriate to generalize this approach and, without abandoning the general criteria, to refine them so that an earlier detection of the resistance development is possible. This could be done by determining the Gaussian distribution of the LC.95 or LT.95 (or better, LC.100 or LT.100) of routinely used insecticides and most probably alternatives for each of the local malaria vectors. One could then statistically determine critical LC or LT values beyond which no susceptible individual would survive. These values could subsequently be used for routine monitoring. Such an approach was developed years ago for culicine mosquitoes and was recently recommended to the Sri Lanka authorities by Dr G. Davidson to drastically improve the sensitivity of malathion resistance monitoring.

It is standard practice to record temperature while carrying out susceptibility tests but this information is rarely used when analysing the test results. Dr C. Ramsdale noticed recently in Turkey that temperatures considerably influence the determination of the LC and LT values whenever carbamate, organophosphates and pyrethroid insecticides are used and this phenomenon was subsequently confirmed during special studies carried out in Dr Davidson's laboratory. This matter should be investigated further with a variety of vectors and insecticides and appropriate guidelines provided to field investigators wherever local climatic conditions require adjustment of the mortality data according to temperature.

The above suggested measures could improve the quality of resistance monitoring at the country and regional levels. It would also be essential to plan this monitoring in such a way that full use be made of what has already been learned regarding resistance development and seasonality of the vector. Efforts should be made to specifically survey areas where agricultural insecticides are profusely applied and to focus the surveys at the time of the year when the vectors are the most abundant; comparative surveys done specifically just before and just after the crop spraying season could be particularly rewarding. Should the country be too large for carrying out a thorough monitoring, efforts could be concentrated on the areas which are the most critical from the malaria control/eradication viewpoint.

Actual potential of alternative insecticides

The above suggested insecticide monitoring surveys should provide a wealth of information regarding the actual potential of alternative insecticides, especially if monitoring activities are carried out with the field trials of new insecticides.

Laboratory cross-resistance studies will also continue to provide useful data on the probable potential of alternative insecticides in relation to the various resistance mechanisms. Furthermore, it is already intended to use more systematically these mechanisms for the laboratory screening of candidate insecticides than has been done in the past.
In view of the limited number of alternative insecticides available at present, one can already note that, from a practical viewpoint:

- the development of resistance to DDT can often prevent the subsequent effective use of pyrethroids;
- the development of resistance to malathion may occur without cross-resistance to other OP insecticides, providing the possibility to use subsequently fenitrothion or similar OP alternatives;
- there is a noticeable risk of development of resistance to IGRs belonging to the methoprene group, but apparently not to diflubenzuron.

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3. THE HUMAN HOST

3.1 Immune phenomena in malaria

The ability of the host to overcome an infection may result from non-immunological factors (innate or natural resistance) or acquired immunity. The relative importance of the two varies throughout the course of infection. Each may change independently from the other so that reduced parasite survival may result from either innate resistance or acquired immunity.

3.1.1 Innate resistance

Innate resistance characteristics are inherited, often as simple Mendelian factors and thus are not elaborated as the direct result of an individual's exposure to infection. In evolutionary terms, however, the selective pressures of malarial infection on human populations profoundly affect the distribution and frequency of traits conferring resistance to the disease. The mechanisms of innate resistance to malaria have only been described against the blood stages.

From studies in Nigeria (Martin et al., 1979) it can be concluded that A, B, C, M, N, S, s, u, Fya, Fyb, Lea, Js and K blood group antigens do not confer a particular advantage or disadvantage as regards infection with P. falciparum. However, susceptibility to infection with P. vivax appears to be closely associated with the absence of Duffy blood group antigens Fya and Fyb (Miller, 1977). This may explain the rarity of P. vivax infections in West and Central African populations. A comparison between P. vivax/P. ovale infection rates in Ethiopians of Nilotic and Hamito-Semitic origin has yielded significantly higher rates in the latter (Armstrong, 1978), but very little difference regarding the frequency of P. falciparum infections. The findings of Lopez-Antunano & Palmer (1978) in Aotus trivirgatus griseimembra suggest that the Duffy group loci are not, by themselves, the site of entry for P. vivax into the erythrocytes. Also the absence of Duffy determinants does not guarantee prevention of invasion since trypsinization and neuraminidase treatment renders Duffy negative erythrocytes susceptible to infection (Miller et al., 1977).

The perpetuation of the sickle cell trait is favoured by P. falciparum. Studies in Nigeria (Fleming et al., 1979; Cornille-Brøgger et al., 1979) showed higher survival rates for haemoglobin (Hb) AS heterozygotes than for Hb A homozygotes in children. At the age of 30-59 weeks, sickle trait carriers showed a lower frequency of infection with P. falciparum and also a lower density of parasites. While the homozygote Hb SS trait appears to be uniformly fatal before the age of 15 years in Nigeria, it shows a rather mild form in Saudi Arabia, with less than 2% annual mortality in Hb SS subjects between 10 and 20 years of age (Perrine et al., 1978), although the underlying selective mechanism, hyperendemic falciparum malaria, seems to be the same. In vitro studies with P. falciparum showed a slight reduction in rates of invasion in AS cells and a marked reduction in SS cells at 5% O2 tension (Pasvol et al., 1978). Growth and multiplication of P. falciparum under high O2 tension (18%) was normal in SS, SA and AA cells, but at O2 tensions of 3-5% inhibition of parasite growth occurred in the SS and SA cells along with sickling. All parasites in SS cells were killed and development in the SA cells stopped at the large ring stage (Friedman, 1978). Parasitized SA cells have an increased tendency of sickling which is apparently associated with a fall of the intraerythrocytic pH following parasite invasion (Friedman et al., 1979) and can be offset by increased potassium levels in the medium. Sickling kills the parasite, but sickled AS cells have a capacity of unsickling after the parasite's death. This results in a population of apparently normal erythrocytes carrying dead parasites.

β-thalassaemia is also associated with resistance to malaria. The persistence of foetal haemoglobin (HbF) is associated with retardation of growth and development of P. falciparum although the mechanism involved is unknown (Pasvol et al., 1977). A lesser degree of inhibition was found with cells containing a mixture of HbF and HbA. In the heterozygote, the switch from HbF to HbA in the first year of life is delayed. This may confer partial resistance to P. falciparum at a time when maternal immunity is waning and active immunity not yet established.
Deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) predisposes erythrocytes to oxidant-induced haemolysis. It has been shown that malaria parasites produce oxygen stress and in G-6-PD deficient erythrocytes, this may result in premature lysis which would release immature parasites incapable of propagating the infection (Eaton et al., 1976). This theory is supported by the production of a similar effect using vitamin E deficiency to increase the oxidant sensitivity of erythrocytes; in addition, vitamin E deficient mice showed increased resistance to P. berghei.

Investigations of erythrocyte G-6-PD and pyridoxal-kinase (PLK) activity in Nigerian children and Caucasian adults suggest that the relatively high frequency of low erythrocyte PLK activity among Nigerians may have been selected for by falciparum malaria (Martin et al., 1978).

3.1.2 Acquired immunity

Apart from innate resistance to malaria, immunity to the disease in many hosts, including man, is acquired only after long exposure to infection and is associated with chronic, low-grade parasitaemia. In endemic areas people may be repeatedly infected throughout life, but the level of parasitaemia and the clinical effects of infection nevertheless tend to diminish with time. In holoendemic areas the classical pattern of malaria infection is characterized by rarity of congenital malaria, resistance to infection in the early months of life, prevalent and dense parasitaemia often associated with severe illness in young children, prevalent but less dense parasitaemia in older children and infrequent low level parasitaemia in adults with clinical illness becoming rare (McGregor, 1974).

The rarity of congenital malaria and infection in young babies is due to passive immunity of maternal origin. As this wanes, young children become highly susceptible and mortality is greatest in children under five years of age. Successive infections induce increasingly effective immune responses, but a sterile immunity is never attained. Immunity is reduced during pregnancy. Outside endemic areas, people of all ages are highly susceptible.

The mechanisms involved in the development of acquired immunity, including immunity stimulated by vaccines are complex and not fully understood. There is now evidence that both cellular and humoral immunity play a role in this process and that non-specific responses may also be important. However, no test has yet been developed which will indicate protection and the development of such a test is of high priority.

The importance of humoral factors against the erythrocytic stages of the malaria parasite has been known since the demonstration of protection of humans by the injection of gammaglobulin from hyperimmune individuals (Cohen et al., 1961). Inhibitory antibody, which in vitro blocks merozoite invasion of erythrocytes (Miller et al., 1975), is species specific and correlates with clinical resistance in a great majority of animals immunized by drug-controlled infections (Butcher & Cohen, 1972) or merozoite vaccination (Butcher et al., 1978). Similarly the level of inhibitory antibody in West African children with P. falciparum malaria usually increases after drug-induced recovery from infection (Wilson & Phillips, 1976).

The importance of humoral immunity in sporozoite immunity has also been demonstrated by the passive protection afforded by antisporozoite antiserum in mice, leading to a reduction in the numbers of exoerythrocytic forms in the liver (Golenser et al., 1978). A major recent advance in this field has been the production of a monoclonal antibody to a single sporozoite surface protein which shows sporozoite neutralizing activity both in vitro and in vivo (Yoshida et al., 1980).

Recent studies have indicated that the cellular response to malarial infection involves both B- and T-cell mediated responses. In experimental animals, it has been shown that T-cells are required for the recovery from malaria infections (Miller & Carter, 1976; Weinbaum et al., 1976). More recently, Cottrell et al. (1978) have demonstrated that delayed cell-mediated hypo- and hypersensitivity reactions which correlated well with protection, were present in vaccinated animals. T-cells may act as helper cells for the provision of specific antibody and they may also be responsible for recruitment of macrophages and natural killer cells (Eugui & Allison, 1979). It has been shown that resistance of different strains of mice to malaria parasites correlates with the degree of Nk cells response to infection (Eugui & Allison, 1979).
Non-specific factors have also been demonstrated to afford protection of mice against \textit{Plasmodium spp.} (Clark et al., 1977).

3.1.3 Serodiagnostic tests

Serodiagnostic tests detect either malaria antigens or antibodies in blood or plasma. The detection of antibodies has its main application in epidemiological studies designed to assess disease endemicity or progress of malaria control operations while antigen-detection tests are more relevant to diagnostic procedures. Field investigations employing many different antibody detection tests have shown that seroepidemiology, as a supplement to the more classical malariometric indices, has much to offer in monitoring the progress of control operations or in assessing the endemicity of malaria in areas where drug treatment is frequent but unrecorded. The development of antigen-detection methods as immunodiagnostic adjuncts has received relatively little attention until recently.

Several methods have been developed for the measurement of malaria antibodies, including indirect immunofluorescence (IFA), haemagglutination (IHA) and complement fixation (CFT). These were compared by Wilson et al. (1975), using sera from patients with naturally-acquired malaria infections and in heroin addicts infected accidentally by needle transmission. Shortly after infection, all tests were equally positive, but several months later the CFT detected fewer positives than the IFA or IHA. False-positive reactions were common in heroin addicts, possibly due to the high level of IgM in these sera. The enzyme-linked immunosorbent assay (ELISA) was developed and applied to malaria serodiagnosis by Voller et al. (1974). This sensitive method has proved useful but may not be efficient for the detection of IgM antibodies. All available serological methods for the measurement of malaria antibodies were reviewed by Voller (1976). Subsequently, a new method was developed by Wilson & Phillips (1976) in which the inhibitory effect of serum antibody on merozoite reinvasion can be assessed in a micro-tissue culture system. Unlike other antibody tests, this method may indicate protective immunity.

Serological methods available for the detection of soluble circulating malarial antigens include gel precipitation, which has been applied epidemiologically by McGregor & Williams (1978), and countercurrent immunoelectrophoresis, which was used by Seitz (1976) to study soluble antigens in murine malaria. Recently, a method has been developed for the detection of malaria parasites in the blood; the test is based on antibody-binding inhibition in a solid-phase assay and has a degree of sensitivity at least equal to that of microscopic examination of thick blood films (Mackey et al., 1980). The methodology was established in a murine malaria model and is currently being adapted to the diagnosis of \textit{P. falciparum}.

It is expected that the studies on antigen isolation and characterization will lead to the development of more sensitive serodiagnostic tests.

3.2 Immunization

Opinion concerning the possibility of developing protective vaccines against malaria has changed notably over recent years. Former pessimism was based on the view that, since natural infections do not induce complete protection, vaccination would be unlikely to do so. However, such pessimism has been substantially reduced following the successful experimental vaccination of animals of several species against malaria, using parasites of different developmental stages. In addition, the establishment of in vitro culture methods for \textit{P. falciparum} has greatly increased the supply of parasites for the production of experimental vaccines. There is now good scientific evidence that protective immunity can be induced by sporozoites and by blood stage parasites and that transmission-blocking immunity can be induced by gametocyte vaccines. Protective immunity has been shown to be both species and stage specific.
Merozoite vaccination using Freund's complete adjuvant (FCA) has proved effective in protecting Macaca mulatta against challenge with P. knowlesi (Mitchell et al., 1975) and Aotus trivirgatus against challenge with P. falciparum (Mitchell et al., 1977). The extreme reactivity of FCA precludes its being considered as a potential clinical adjuvant in malaria vaccination and therefore studies have been conducted to find a suitable replacement. A muramyl dipeptide derivative given in mineral oil has proved to be partially effective as an adjuvant in merozoite vaccination of M. mulatta against P. knowlesi and saponin is effective in similar vaccination of M. fascicularis (Mitchell et al., 1979). Also a stearoyl muramyl dipeptide in liposomes has been shown to be effective with a preparation of saponin-lysed P. falciparum schizonts in protecting Aotus monkeys against a lethal homologous challenge (Siddiqui et al., 1979). The studies of Welide et al. (1979) with irradiated merozoites and schizonts indicate that protection can be obtained without the use of adjuvants.

It is likely that parasites grown in vitro will be a fundamental requirement for any merozoite vaccine either directly through mass cultivation or indirectly as a basis for analytical studies. Immunization against malaria with antigen from continuous in vitro cultivated P. falciparum has been demonstrated (Reese et al., 1978).

Immunization of mice with irradiated sporozoites results in protection of more than 90% of these animals against an otherwise lethal challenge with viable sporozoites (Nussenzweig, 1977). Effective immunization has also been observed in a small number of human volunteers inoculated with irradiated sporozoites of both P. falciparum (Clyde et al., 1973; Rieckmann et al., 1974a) and P. vivax (Clyde et al., 1975). The observations in mice have also been validated in a simian model with P. knowlesi (Gwadz et al., 1979b). Protection in this model is significant since there is otherwise a very acute infection and the finding that multiple injections of sporozoites appear necessary to achieve a protective immunity of several months duration is similar to that observed in rodents. Only the intravenous route is effective in inducing both protection and antisporozoite antibodies and recent studies show that the mechanism of resistance to sporozoites probably involves the interaction of the host's immune system with the parasite's surface (Gwadz et al., 1979b; Yoshida et al., 1980).

The possibility of developing gamete vaccines which would block transmission and which could be used for malaria control has become apparent from work performed in the late 1970s. These studies with avian and primate models have demonstrated that immunization of the host with extracellular gametes totally suppresses infectivity of the mosquito at a subsequent blood meal. Gametocytes within the erythrocytes are unaffected by the immunity, since resuspending the gametocytes in serum from normal non-immune animals restores their infectivity to mosquitoes. Immunity is mediated by antibodies that are ingested with the blood meal. These antibodies interact with extracellular gametes and prevent fertilization (the fusion of male and female gametes). Thus the infection in the mosquito is blocked, and in this way transmission is interrupted (Gwadz, 1976; Carter et al., 1979; Gwadz et al., 1979a).

Gamete immunization of monkeys but not of chickens is dependent on the use of an adjuvant of which FCA is at present the only fully effective one. Mendis & Targett (1979) have recently reported the successful immunization of mice against the sexual stages of P. yoelii using formalin-fixed parasites and no adjuvant.

The production of a malarial vaccine could be from intact parasites, parasite fractions or purified antigens. Although considerable progress has been made in the separation of parasite stages (see section 1.3), the problem of obtaining parasites in a pure form, free from contamination of host cell debris and extraneous material, has not been overcome. There has therefore been increasing interest in recent years in the possibility of immunizing with purified parasite antigen rather than with intact parasites of any developmental stage.

Major impetus has come from the demonstration that protection against P. gallinaceum can be induced with a single histidine-rich surface protein (Kilejian, 1978). Highly significant work on the production of a monoclonal antibody to a single surface protein of P. berghei sporozoites which proved to have sporozoite neutralizing activity both in vitro and in vivo (Yoshida et al., 1980) illustrates the value of hybridoma-produced monoclonal antibodies in malaria research. Such antibodies are currently being developed for the identification and purification of protective antigens for use in vaccine experiments.
3.3 Pathology (including immunopathology)

Two major factors determine the pathology of malaria, regardless of the parasite species involved:

(i) the multiplication of parasites and linked with it the destruction of infected red blood cells, and the liberation of malarial pigment;

(ii) cellular and humoral host responses which may lead to the excessive destruction of non-infected erythrocytes, formation of immune complexes and their disposition in various tissues, and other pathological phenomena.

In falciparum malaria, an additional factor is the adhesion of infected red blood cells to the capillary endothelium; this phenomenon is associated with the appearance of knob-like protrusions on the infected erythrocyte (Langreth et al., 1978), it is also to a significant extent responsible for specific complications of falciparum malaria such as cerebral malaria and algid malaria. Nutritional factors were suspected to have an additional bearing on the severity of malaria.

While studies on the macroscopic and histological pathology of malaria, especially studies related to the appearance of pigment in tissues, commenced in Meckel's era in the mid-nineteenth century, it was only in the twentieth century that immunological factors began to be investigated as the cause of pathological phenomena in malaria. Such studies have been successful in demonstrating and elucidating the immunopathological character of the nephrotic syndrome in P. malariae infections. However, the role of immunosuppression and its inter-relation with the response to pathogens other than plasmodia, the causation of excessive destruction of non-infected erythrocytes, the occurrence of pulmonary oedema and the tropical splenomegaly syndrome (TSS) remained enigmas for a long time and it is only very recently that they have begun to be solved.

Nutritional factors appear to influence the development of cerebral malaria. Sahelian Fulani children surviving on dairy produce alone did not suffer from cerebral malaria, whereas Kanuri children from the same area, living on millet, had a significant incidence of this form of malaria (Murray et al., 1978). Studies of Moore et al. (1977) showed that acute protein energy malnutrition alters proliferative responses of lymphocytes to phytohaemagglutinin (PHA), indicative of important alterations of the immune response.

Studies in 80 Indian infants and young children suffering from vivax malaria showed splenomegaly in 83%, hepatomegaly in 68%, and jaundice in 9% of patients. Serum aspartate transaminase (AST) was raised in 66%, serum albumin transaminase (ALT) in 39% and alkaline phosphatase in 46% of all cases. AST and ALT levels were highest in patients with hepatomegaly (Patwari et al., 1979). Similarly, the activity of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) in the plasma of P. berghei infected albino mice was increased, while there was a significant decrease of activity of these enzymes in the liver during high parasitaemia (Lal & Hussain, 1978),

The metabolic alterations are also reflected in gross modifications of the protein levels.

Acute falciparum malaria raises the levels of total plasma protein, \( \xi \)-1-glycoprotein and 1-post albumin peak as assessed by polyacrylamide electrophoresis. The latter two were decreased some four weeks after treatment. Transferrin was significantly reduced and remained so for some considerable time (Migasena et al., 1978).
The renal lesions in malaria have been studied on a long-term basis and it has been shown that two distinct syndromes occur (Houba, 1975). An acute, transient glomerulonephritis develops shortly after *P. falciparum* infection, when malarial antigens, antibodies and complement are demonstrable in the glomeruli. The condition responds to antimalarials. Similar lesions have been found in rhesus monkeys infected with *P. cynomolgi* (Voller, 1975) and in mice infected with *P. berghei* (George et al., 1976). Occasionally, this transient glomerulonephritis may lead to acute renal insufficiency as recently reported by Bourdais et al. (1978) in the case of gravidae near term. The second syndrome is characterized by the development of chronic progressive lesions in association with *P. malariae* infection. There is glomerular deposition of IgG and IgM, specific antibody, *P. malariae* antigens and complement. Nephrotic syndrome supervenes, and this does not respond to antimalarials. It appears that immune complexes are involved in the pathogenesis of both forms of nephropathy, but that other factors, possibly including auto-antibodies, also contribute to the development of chronic lesions.

Tropical splenomegaly syndrome (TSS) develops in people who appear to have an abnormal immune response to repeated malaria infections. Genetic factors are involved in predisposing to TSS (Fakunle & Greenwood, 1976; Greenwood, 1979; Crane, 1979). Affected individuals show excessive IgM production and the formation of large molecular weight immune complexes, which are phagocytosed in the liver and spleen leading to the characteristic hepatic and splenic changes. It is possible, though not certain, that the immune complexes also adhere to the surface of red blood cells, thereby promoting their destruction and contributing to the development of anaemia.

Immunological factors have been suspected for quite some time to play a role in the etiology of malarial anaemia. In Nigeria, a small proportion of both anaemic and non-anaemic children had sensitized erythrocytes and anaemia did not appear to be related to the presence of circulating immune complexes or to any other immunological factor (Greenwood et al., 1978; Greenwood & Fakunle, 1979). Studies of Woodruff et al. (1979) demonstrated, by chromium release, a significantly shortened half-life of erythrocytes from patients suffering from falciparum, vivax and ovale malaria. The reduced half-life was associated with the presence of complement-containing immune complexes on the red-cell surface. $^{59}$Fe studies of erythropoiesis indicated a mild degree of marrow depression. Thus it appears that a complement-mediated process is an important factor causing anaemia in malaria.

Pulmonary oedema is a severe, often fatal complication of falciparum malaria and appears to be a result of altered permeability of the capillary membrane. Recent reports (Marks et al., 1977; Leelarasamee et al., 1978; Fein et al., 1978) indicate improvements in the management of this condition.

Controversy still exists regarding diffuse intravascular coagulation (DIC) in falciparum malaria. Anuar & Yap (1979) described a case with typical DIC phenomena, but Vreeken & Cremer-Goote (1978) came to the conclusion that none of their patients with similar symptoms had a true DIC as characterized by an activation of the fibrinolytic system, an activation of the clotting mechanism, and the consumption of platelets, fibrinogen and factor V. They therefore concluded that heparin treatment is rarely indicated in patients with severe falciparum malaria.

Malarial infections are known to depress immune responses to diverse antigens in animal models (for review see Voller, 1975) and in humans (Greenwood et al., 1972; Williamson & Greenwood, 1978). In these latter studies, children with malaria had lower induced responses to *Salmonella typhi* O antigens, tetanus toxoid and meningococcal vaccines. In the case of meningococcal vaccines, the suppression persisted for at least a month after treatment (Williamson & Greenwood, 1978). These studies indicate that there is a deficiency in antigen handling occurring in *P. falciparum* infections similar to that reported in animal models. The complete mechanism is unknown but it appears to result from a functional defect of the adherent spleen cells which affect antigen uptake (Warren & Weindans, 1976) and handling by macrophages (Tanabe et al., 1977).
Conversely, simultaneous infection with viruses may exacerbate the malaria attack as shown with dehydrogenase-elevating virus (LDV) in mice infected with *P. yoelii* (Henderson et al., 1978). This may be of particular interest for the interpretation of experimental results in the *P. yoelii*/mouse model since LDV infections in laboratory animals are a distinct possibility. Another epidemiologically important interaction between malaria and virus diseases is Burkitt's lymphoma. Based on a review of 133 cases, Aderele & Antia (1979) postulate that an interaction between malaria, EB virus infection and malnutrition are important factors in the etiology of the tumour.

Precisely how the malaria parasite achieves its immunopathological effect is imperfectly understood but there is increasing evidence which indicates that in many instances it is the formation of immune complexes which may trigger the subsequent sequence of events. The appearance of immune complexes has been shown to follow that of soluble antigen and specific antibody and to be associated with profound depletion of complement components and deposition of complexes in the tissues (Perrin et al., 1979; June et al., 1979). New methods have recently been developed for the isolation of immune complexes from serum (Casali et al., 1977) and these are being applied to the analysis of malaria-associated complexes. Partial characterization of such complexes has been carried out by ultracentrifugation on sucrose density gradients (June et al., 1979). Further work is in progress on the identification of antigen specificity in immune complexes in malaria and on evaluating the role of these immune complexes in the pathogenesis of tissue lesions.

3.4 Chemotherapy

3.4.1 Drugs in current use

Although chloroquine resistance of *P. falciparum* has seriously restricted the usefulness of this drug in eastern Asia and southern America (see also 3.4.3), the medicament is still rendering good results against *P. falciparum* in other parts of the world. It has remained fully effective against *P. vivax* around the world.

Recently attention has been mainly focused on side-effects of chloroquine (see 3.5.1), but some studies have also covered the operational use of the drug. Thus, Chayabeja et al. (1975) used chloroquine for suppression at fortnightly or monthly doses of 10 mg base/kg body weight in schoolchildren of a holoendemic area of Zambia where *P. falciparum* is the predominant species. Under the fortnightly regimen the parasite rate fell within four months from 47% to 0%, under the monthly regimen from 76% to 15%, with a disappearance of *P. ovale* and a drastic relative reduction of *P. malariae*. During the same period the control groups showed no major change or a rise of the parasite rates.

In Tamil Nadu, India, treatment with a single dose of 600 mg chloroquine (base) cleared 99% of *P. vivax* infections (476) and all *P. falciparum* infections (33) by the seventh day after treatment (Roy, 1978).

Chloroquine is considered to be a blood schizontocidal drug only. Smalley (1977) showed that it also interrupts gametocytogenesis up to the sixth day, while there is no impact on growing gametocytes after the sixth day. Thus, *P. falciparum* infections treated early in the primary attack may not develop any gametocytes at all.

A comparison between the activities of various 4-aminoquinolines in chloroquine-resistant *P. falciparum* in Aotus (Schmidt et al., 1977c) showed that such strains were more susceptible to amodiaquine, amopyroquine, dichlorquinazine, SN-8137 and SN-9584 than to chloroquine. Clinically acceptable higher doses of these drugs were able to produce radical cure in chloroquine-resistant infections.

A new, simple gas chromatographic assay method of chloroquine in biological fluids, with a sensitivity of 0.1 n-mol/ml has been introduced by Viala et al. (1978).
Chloroquine activity is potentiated by erythromycin in chloroquine-resistant *P. berghei*, while the effect of both drugs is only additive in chloroquine-sensitive *P. berghei* (Warhurst et al., 1976). This may be explained by an increased permeability of the mitochondrial membrane to erythromycin in the presence of very high chloroquine concentrations as seen in chloroquine-resistant parasites (Warhurst, 1977) which would admit increased amounts of the antibiotic.

Combination of long-acting sulfonamides and pyrimethamine are widely used as alternative drugs for the treatment and suppression of infections with chloroquine-resistant *P. falciparum*. Schmidt et al. (1977b) reported that sulfadiazine and pyrimethamine, if used in combination, show a significant enhancement of the activities of the single compounds which amounts to a 32-fold increase of pyrimethamine activity and a 50-100-fold increase in sulfadiazine activity.

The combination of sulfadoxine and pyrimethamine is the most widely used alternative medication in areas with chloroquine-resistant *P. falciparum*. In a field study in Thailand, Pearlman et al. (1977a) observed an eightfold reduction of *P. falciparum* parasitaemias and a threefold reduction of *P. vivax* parasitaemias when 1000 mg sulfadoxine and 50 mg pyrimethamine were given every two weeks. Half this dose and also diformylapsone (DFD) 400 mg with 25 mg pyrimethamine every two weeks were less effective. The high-dose group under sulfadoxine and pyrimethamine showed a slight but statistically significant decrease in the number of leucocytes. Treatment with the sulfadoxine-pyrimethamine combination (1500 mg SD + 75 mg P adult dose) of falciparum malaria in Laos produced parasite clearance in all cases, but by day 7 gametocytes were still present in 45% of all cases.

A treatment study with pyrimethamine (50 mg) versus sulfadoxine and pyrimethamine (100 mg SD + 50 mg P or 1500 mg SD + 75 mg P) in patients with vivax malaria in Thailand (Doberstyn et al., 1979b) showed that pyrimethamine alone was less effective, clearing only one-third of the infections by day 7, while three-fifths of the infections were cleared by the lower dosed combination. The higher dosed combination produced clearance in all patients, but the mean clearance time was relatively long (90 hours). Chloroquine remains the drug of choice for the treatment of acute vivax malaria.

Studies of Eling & Jerusalem (1977) throw an interesting light on the effects of sulfonamide in the *P. berghei/mouse* model, where prolonged suppression with sulfathiazole produced effective immunity.

Clindamycin is occasionally used as a third-line alternative drug for the treatment of chloroquine- and sulfadoxine-pyrimethamine-resistant falciparum malaria. In the *P. knowlesi/Macaca mulatta* model the drug and its N-demethyl-4'-pentyl analogue showed considerably slower action as compared to chloroquine (Powers et al., 1976).

The radical treatment of vivax malaria poses major problems in the field. The need for administering a fortnight's course and the side-effects, especially in G-6-PD deficient persons, are major obstacles to the use of primaquine. Schmidt et al. (1977a) observed that the radical curative (tissue schizontocidal) effect of primaquine is a function of total dose rather than the duration of administration, by comparing regimens of various duration (with the same total drug dose). This throws some doubt on the efficacy of the five-day regimen with 15 mg primaquine per day as used in India, although Roy et al. (1977) observed a relapse rate of only 1.3% in 6393 vivax cases so treated. Studies in El Salvador (Cedillos et al., 1978) indicate a higher relapse rate than that observed in India, using amodiaquine as a schizontocide and a five-day course of primaquine at 15 mg per day.

A comparison between the administration of 1500 mg chloroquine over three days plus 75 mg primaquine over three days and the weekly administration of 300 mg chloroquine and 45 mg pyrimethamine for 12 weeks in *P. vivax* infections in the Solomon Islands (Saint-Yves, 1975) demonstrated that the weekly regimen produced better results with a subsequent attack rate of 5.4% as against 12.2% under the five-day regimen.
For the radical treatment of vivax malaria in G-6-PD deficient persons Trenholme & Carson (1978) recommend a standard weekly regimen of 300 mg chloroquine and 45 mg primaquine for the duration of eight weeks in patients of African origin, while weekly doses of 300 mg chloroquine and 30 mg primaquine for a total duration of 15 weeks should be given to patients of Mediterranean or Asian origin.

3.4.2 Drugs under development

Since the development of the currently used 4-aminoquinolines and dihydrofolate reductase inhibitors, relatively little antimalarial drug research has been carried out by the pharmaceutical industry. Only the United States Army, through the Walter Reed Army Institute of Research, has maintained a major programme in which some 300 000 compounds have undergone primary screening (Richards, 1979). Approximately 3% of the compounds showed activity in the primary screen, but only a fraction of these qualified eventually for further preclinical or clinical development. Such compounds were found in the groups of 4,6-diamino-1-substituted dihydrotriazines, naphthoquinones, 9-phenanthrenemethanols, 4-quinoline-methanols, 4-pyridinemethanols, arylthio-quinazolines and phenylphenols. Recently plant-derived sesquiterpene lactones received considerable attention in China.

In the following an attempt is made to summarize information on the most promising compounds which have reached the stage of advanced preclinical or clinical development.

Among the 4-quinolinemethanols, mefloquine is the most advanced drug, being in the stage of phase I, II and III clinical trials. The compound is a highly effective schizontocide which is also active against infections with chloroquine-resistant \textit{P. falciparum}. Mefloquine is also highly effective against blood forms of \textit{P. vivax}, but it has no effect on exoerythrocytic forms. It is generally well tolerated at therapeutic doses. The curative dose level for \textit{P. falciparum} appears to be situated at approximately 15 mg base/kg body weight. The mode of action of mefloquine resembles that of quinine, but its activity in chloroquine-resistant \textit{P. berghei} is less than in chloroquine sensitive strains (Peters et al., 1977a, b). Mixtures of mefloquine with pyrimethamine or sulfaphenazole have an additive effect. Volunteer studies on the prophylactic effect of mefloquine (Rieckmann et al., 1974b) showed that a single dose exerted prolonged suppressive activity against a chloroquine-resistant strain of \textit{P. falciparum}, an observation which was confirmed in a field trial in Thailand (Pearlman et al., 1977b). This is explained by the long half-life of mefloquine, ranging from 22 to 33 days in Caucasians (Schwartz et al., 1980). The comparison of single dose treatment with 1.5 g mefloquine hydrochloride or with 1500 mg sulfadoxine plus 75 mg pyrimethamine in adult Thais with falciparum malaria showed radical cure in all 37 patients under mefloquine and in 34 out of 38 cases treated with the sulfadoxine/pyrimethamine combination (Doberstyn et al., 1979a).

Two further 4-aminoquinolines, coded compounds WR 184 806 and WR 226 253, studied in the \textit{P. falciparum/Aotus} model were, respectively, one-third and twice as active as mefloquine (Schmidt et al., 1978b).

9-phenanthrenemethanols, especially 3,6-trifluoromethyl-\(\alpha\)-(2-piperidyl)-9-phenanthrenemethanol (WR 122 455), hold considerable promise for further development. Experimentally, in the \textit{P. berghei}/mouse model, WR 122 455 proved to be highly active against primaquine-, sulfonamide-, pyrimethamine-, cycloguanil- and moderately chloroquine-resistant lines. It was inactive against a highly chloroquine-resistant line (Peters & Porter, 1976). In contrast to other 9-phenanthrenemethanols, WR 122 455 has an acceptable chemotherapeutic index, but it needs a higher dose level than chloroquine. It is a blood schizontocide and has apparently no tissue schizontocidal action (Porter & Peters, 1976). Volunteer studies with WR 122 455 and WR 171 669 (\(-1\)-(1,3-dichloro-6-trifluoromethyl-9-phenanthryl)-3-di(n-butyl)aminopropanol HC1) given orally against infections with chloroquine-sensitive and chloroquine-resistant \textit{P. falciparum} produced encouraging results. Single dose treatment with WR 122 455 cleared parasitaemia, but recrudescences occurred. However, six-day treatment with 240 mg WR 122 455 twice daily produced S responses in all chloroquine-sensitive and chloroquine-resistant infections (Rinehardt et al., 1976). Studies in the \textit{P. falciparum/Aotus}}
system showed that WR 122 455 was four times as active as chloroquine and that WR 171 699 was as effective as chloroquine against a sensitive strain. In this system both compounds were also active against chloroquine-, pyrimethamine- and quinine- mono- or multi-resistant strains (Schmidt et al., 1978a).

WR 194 965 and WR 204 165, both α-amino-o-cresol derivatives, represent a new generation of candidate compounds. Schmidt & Crosby (1978) found the level of activity of both drugs similar to that of mefloquine, but WR 194 965 was selected for further studies since WR 204 165 appears to be converted into WR 194 965 through in vivo cleavage. WR 194 965 was effective against the chloroquine-, pyrimethamine-, quinine-resistant P. falciparum Viet Nam Smith strain in Aotus (Schmidt & Crosby, 1978).

WR 158 122 [2,4-diamino-6-(2 naphthyl sulfanyl) quinazolinel and its tetrahydro analogue WR 180 872 have also reached a preclinical development stage. Both compounds appear to be dihydrofolate reductase inhibitors. They potentiate the antimalarial activity of sulfadiazine (Kinnamon et al., 1976).

The first antimalarial drug used in the pure form is quinine, a compound extracted from Cinchona. In 1972, a plant-derived antimalarial compound was extracted from Artemisia annua L. in China, where the plant has traditionally been used over 2000 years for the treatment of intermittent fever. Since 1972, extensive work has been done with this compound, named Qinghaosu, and this has been summarized by the Qinghaosu Antimalaria Coordinating Research Group (1979). Qinghaosu is a sesquiterpene lactone, the structure of which has been identified. Its toxicity is low with an LD50 of 5105 mg/kg (orally) in the mouse and 1558 mg/kg intraperitoneally. It has a blood schizontocidal effect and little or no action on gametocytes and exoerythrocytic forms. The half-life of the drug in mice was four hours only, some 80% of the drug was eliminated through urine and stools within 24 hours. Clinical studies in which Qinghaosu was administered to 1511 patients with vivax malaria, 558 with P. falciparum infections, 141 with cerebral malaria and 143 with chloroquine-resistant falciparum malaria, yielded promising results. Fever in the P. vivax infections subsided within 30-40 hours. Some 10-30% of the P. vivax cases and 10-25% of the P. falciparum cases recrudesced, depending on dose level and formulations used. The best results were obtained with oil or water suspensions of Qinghaosu. The recommended regimen is a three-day course of 300-400 mg Qinghaosu (total dose 0.9-1.2 g) as intramuscular injections of oil or water suspension. Chloroquine-resistant P. falciparum and cerebral malaria responded well to the drug, with results comparable to those obtained with quinine.

In this connexion it is interesting to note that an aqueous extract of Azadirachta indica leaves has a schizontocidal action on chloroquine-sensitive P. berghei (Ekanem, 1978).

Recently, considerable efforts have been made in the area of improving galenic formulations of antimalarial drugs and the production of sustained release systems. However, as much of this development is carried out in industry though often in collaboration with WHO, much of the data are subject to confidentiality and little material is published before the systems have reached operational maturity. The same applies to certain sectors of drug development, e.g. triazines, which are therefore not covered in this review.

The field of biodegradable matrices for the sustained release of antimalarial drugs has made progress with the development of a polylactate-polyglycolate copolymer matrix which was used for the incorporation of sulfadiazine and the diaminoquinazoline WR 158 122, obtaining sustained release of the drugs at active level for 13 weeks (Wise et al., 1979).

Attempts are also being made to reduce the toxicity of primaquine by developing techniques to target the drug to the parasite and thereby increase its efficacy and reduce its toxicity. The results show that primaquine entrapped within cholesterol-rich negatively-charged, small multi-lamellate liposomes is considerably less toxic than free primaquine. Drug efficacy is also improved (Pirson et al., 1979). However, Alving et al. (1979) prevented the development of erythrocytic parasitaemia by the intravenous injection of liposomes.
containing neutral glycolipids with a terminal glucose or galactose to mice previously infected with sporozoites of *P. berghei*. Liposome, entrapped glycolipids did not inhibit development of parasitaemia when the mice were infected with erythrocytic parasites.

3.5 Drug resistance

The introduction of the standard *in vitro* test for the assessment of chloroquine and mefloquine sensitivity in *P. falciparum* has produced a wider application of this test, especially in eastern Asia and South America. Valera & Shute (1975) validated this test system in comparison with the results of the WHO standard *in vivo* test (extended 28-day test). The development of the continuous *in vitro* cultivation of *P. falciparum* by Trager & Jensen (1976) has also brought about an improvement of *in vitro* drug sensitivity testing. Rieckmann et al. (1978) described a micro-technique which they used for the assessment of chloroquine and mefloquine sensitivity in blood from *Anotus* infected with *P. falciparum*. Subsequently, the method was further developed and, after validation in Thailand, employed for studies in Brazil, Colombia, and Sudan (Lopez-Antuñano & Wernsdorfer, 1979; Kouznetsov et al., 1979). The micro-method, using a growth medium similar to that employed in continuous culture, requires only small quantities of blood, obtainable through skin puncture, is largely independent of the growth stage of the rings of *P. falciparum* and produces adequate control growth in the large majority of tests with material containing more than 500 asexual parasites per microlitre blood. Specific adsorption of drugs to surfaces of test plates or of materials used in their preparation has been recognized as a problem in the standardization of the test, but it can be overcome since the pattern of adsorption is regular. There is a definite correlation between parasite and chloroquine quantity at parasite densities exceeding 100 000 rings per microlitre blood, apparently caused by specific uptake of the drug by the parasite (Wernsdorfer, 1980). The results of macro- (standard) and micro-tests show very close resemblance. The micro-test is suitable for testing with 4-aminoquinolines, mefloquine, quinine and Qinghaosu.

A susceptibility test system for 4-aminoquinolines and dihydrofolate reductase inhibitors in a continuous culture system of *P. falciparum* has been developed by Richards & Maples (1979). This system uses growth/inhibition impact on parasite viability as critical parameters, while the above-mentioned field tests measure the inhibition of schizont maturation.

In recent years studies of chloroquine response of *P. falciparum* have been reported from Bangladesh (Rosenberg & Maheswary, 1976), Burma (Tin & Hlaing, 1977), India (Kunte & Mitra, 1978; Dwivedi et al., 1978; Rooney, 1979), Indonesia (Verdrager et al., 1975a, b), Papua New Guinea (Simpson & Williams, 1978; Han & Grimmond, 1976), Philippines (Valera & Shute, 1975), Thailand (Sucharit et al., 1977a, b), Ethiopia (Armsrong et al., 1976; Palmer et al., 1976) and Brazil and Colombia (Lopez-Antuñano & Wernsdorfer, 1979). The global collaborative studies on assessment and monitoring of drug response of malaria parasites, conducted in the framework of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, has made good progress with implementation in the Western Pacific, South-East Asia and American Regions. Susceptibility data are regularly published in the *WHO Weekly Epidemiological Record*. The latest issue (WHO, 1979c) shows the following countries to be affected by chloroquine-resistant *P. falciparum*:

**SEAR:** Bangladesh, Burma, India (North-East and Orissa), Indonesia (Kalimantan, West Irian), and Thailand;

**WPR:** Kampuchea, Laos, Malaysia (Peninsular and Sabah), Papua New Guinea, Philippines and Viet Nam;

**AMR:** Brazil, Colombia, Ecuador, French Guiana, Guyana, Suriname and Venezuela.

Chloroquine-resistant *P. falciparum* occurs also in China (Qinghaosu Antimalaria Coordinating Research Group, 1979).

Reports of chloroquine-resistant *P. falciparum* infections in Africa caused much concern. **RI resistance has been substantiated in four cases, non-immune Caucasians, who had contracted**
the infection in Kenya (3) or in Tanzania (1). All cases responded with radical cure to alternative treatment (Fogh et al., 1979; Kean, 1979; Communicable Disease Center, 1978; Stille, 1979). One of the isolates designated as Tanzanian I/CDC has been inoculated into two Aotus monkeys. The infection recrudesced after a dose of chloroquine of 40 mg/kg which would normally cure the infection with a susceptible strain. The 48-hour in vitro chloroquine sensitivity test indicated that Tanzanian I/CDC isolate has a similar resistance pattern to the Viet Nam Oak-Knoll strain (Campbell et al., 1979).

In this connexion it is interesting to note that Kouznetsov et al. (1979) found P. falciparum from the Sennar area, Sudan, to be less susceptible to chloroquine than in most other African countries, although in vivo response was uniformly of the S type. The in vitro levels of complete inhibition of schizont maturation are indeed close to the critical levels of incipient resistance, resembling those observed in Ethiopia by Armstrong et al. (1976) and Palmer et al. (1976) in an area so far exposed to very little drug pressure. The isolates from Sennar showed a high degree of homogeneity in drug response. It is therefore likely that the relatively low sensitivity of the Sennar isolates represents innate strain characteristics rather than a sign of strain selection under drug pressure.

Lopez-Antuñano & Wernsdorfer (1979) found schizont maturation in P. falciparum of medium-high resistance to chloroquine to be completely inhibited by relatively low mefloquine concentrations within therapeutic range, at approximately one-seventh to one-eighth of the substance concentration required for chloroquine.

The first published report of refractoriness to fannedr treatment of P. falciparum came from Indonesia (Ruemans et al., 1979). A 60-year-old individual, although receiving chloroquine prophylaxis at 300 mg base twice weekly, developed malaria in Irian Java. As he relapsed, quinine sulfate at a dose of 3 x 2 tablets of 222 mg each was taken daily by the patient as self-medication. Since the patient remained acutely ill, fannedr (1500 mg sulfadoxine + 75 mg pyrimethamine) was administered. The parasitaemia cleared within 48-72 hours, but 27 days later a recrudescence occurred and parasitaemia reappeared.

A. stephensi and A. balabacensis balabacensis proved to have very similar susceptibility to infection with chloroquine-resistant P. falciparum from Thailand (Sucharit et al., 1977b).

3.6 Toxicological aspects

3.6.1 Anti-malarial drugs

The subject of toxicity and side-effects has recently been extensively reviewed under the auspices of WHO (Weniger, 1979a, b, c).

Chloroquine, the main representative of currently used 4-aminoquinolines, is one of the most widely used antimalarials since some 30 years. Its toxic effects depend on dosage and duration of administration, and these in turn depend on the condition under treatment. Most of the side-effects are actually associated with its use in the treatment of collagen diseases where chloroquine is administered in much higher doses than those usually applied in the suppression of malaria. This applies particularly to toxic phenomena, e.g. retinopathy, which are apparently caused by the accumulation of the drug and its specific binding to melanin. Surprisingly little experimental and clinical data have been published in the course of the last five years while general discussion about retinopathy and its relation to malaria prophylaxis has continued unabated.

Among the minor side-effects pruritus seems to be one which restricts the use of chloroquine for malaria prophylaxis in Nigeria (Olatunde, 1977). An unusual toxic reaction, consisting in chloroquine-induced involuntary movements, has been reported by Umez-Eronini & Eronini (1977) from Nigeria; similar symptoms were previously described under amodiaquine by Akindele & Odejide (1976). Gupta (1977) reported haziness of vision and abnormal eye movements.
Acute toxic effects of single doses are usually associated with accidental intake of overdoses, mainly in children, or with the intention of committing suicide. Whereas reports on accidental poisoning have decreased, chloroquine seems to be a much used drug for suicidal attempts, especially in Africa. Between 1955 and 1978 some 335 cases of voluntary chloroquine poisoning have been reported (Weniger, 1979b), 135 of which resulted in death. The fatal doses ranged between 1 and 26.7 g of base. Acute chloroquine poisoning provokes neurological, respiratory and cardiovascular symptoms.

Acute toxic symptoms are rarely associated with the use of therapeutic doses of chloroquine, but acute cardiovascular shock is known to occur especially in children after parenteral administration of the drug. Intravascular haemolysis leading to acute renal insufficiency occurred in three G-6-PD deficient children from India, following the curative administration of chloroquine. All patients recovered (Choudhry et al., 1978). Fletcher & Sarikhabuti (1978), however, conclude that the antimalarial activity of chloroquine does not appear to be associated with erythrocytic G-6-PD.

Among the chronic complications, occurring under high doses used for the treatment of collagen diseases, keratopathy and retinopathy are the most important (Weniger, 1979b). Keratopathy is a reversible condition caused by the deposition of granular, opaque material in the corneal epithelium, probably chloroquine or its metabolite. It causes temporary visual defects, but is always reversible after the withdrawal of the drug.

The occurrence of retinopathy is apparently a function of total cumulative dose, duration of chloroquine intake and age (Elman et al., 1976), but there is no report of retinopathy having occurred under cumulative doses of less than 100 g chloroquine base.

Retinopathy occurring after the use of chloroquine for malaria prophylaxis has only been described from francophone countries, where usually 100 mg chloroquine base is taken daily for this purpose (Weniger, 1979b). There are seven pertinent case histories (Fauqique et al., 1964; François & Becker, 1965; Ravault, 1965; Trojan, 1975) with a total cumulative intake of 365-730 g. There is no evidence that chloroquine-associated retinopathy has ever occurred under prophylactic doses of 300 mg weekly (Weniger, 1979b).

The use of chloroquine in normal malaria prophylactic doses does not seem to cause ototoxicity (Weniger, 1979b), but Dencker & Lindquist (1975) observed a heavy accumulation of chloroquine in the inner ear of pigmented rats, with a high degree of retention after discontinuation of the drug.

Myopathy (neuromyopathy) under chloroquine does not occur under the chloroquine dose used for malaria prophylaxis (Weniger, 1979b).

A case of complete heart block was observed in a Liberian patient, who had taken approximately 150 mg chloroquine base daily for about three years (Edwards et al., 1978).

There is no evidence that normal malaria prophylactic doses of chloroquine taken during pregnancy would cause teratogenic effects (Weniger, 1979b).

Nagaratman et al. (1978) reported on three cases of aplastic anaemia associated with long-term or short-term administration of chloroquine.

Very few new reports have become available on pyrimethamine toxicity. Acute poisoning, almost exclusively in children, has become rare. Sixteen out of the 28 cases in the literature were fatal (Weniger, 1979c), convulsions were the main symptom. The fatal doses ranged between 250 and 1625 mg, approximately 10-69 times the recommended weekly prophylactic dose for the appropriate age groups.

Chronic toxicity is mainly associated with high daily doses of pyrimethamine such as those required for the treatment of toxoplasmosis. The principal toxic effects are gastro-
intestinal disturbances and bone marrow depression and its sequelae, especially in folate-deficient subjects.

New data on primaquine and other 8-aminoquinolines are also scarce. Strother et al. (1975) studied the metabolism of primaquine in dogs and found a blood concentration peak within two hours of administration. Four metabolites were isolated from the urine. Methaemoglobinaemia under primaquine appears to be associated with metabolites and not the parent compound.

Primaquine and other 8-aminoquinolines may provoke nausea, vomiting, anorexia, dizziness, epigastric distress and abdominal pain or cramps, but haematological side-effects are more prominent (Weniger, 1979a). Neutropenia and agranulocytosis are usually only associated with an overdose of primaquine, but formation of methaemoglobin and haemolysis are more frequent occurrences, especially in G-6-PD deficient subjects (see also 3.3).

Diformylaminodiphenylsulfone (DFD) also causes haemolysis in G-6-PD deficient subjects as Poshyachinda et al. (1978) observed in six out of 12 G-6-PD deficient volunteers in Thailand.

3.6.2 Insecticides and larvicides

Due to some reluctance from the industry to put resources into research on new pesticides, there have been few substantial changes during the last five years in pesticides used in public health except for the emergence of the synthetic pyrethroids and insect growth regulators.

Antimalaria programmes are the main consumers of insecticides in public health. The extension of vector resistance led to the use of a greater variety of compounds. The new compounds must however be cheap, effective and safe. The safety aspect is essential particularly for insecticides used indoors as residual spray when it is not practicable to reduce human exposure either for spraymen or inhabitants. Therefore compounds of greater than moderate hazard are classified in the WHO Recommended Classification of Pesticides by Hazard (PDS 1978a). The World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations distributes Data Sheets on Pesticides which give information on chemistry, use and toxicology of about 50 pesticides, including most used in malaria control.

It is moreover known that small amounts of certain impurities in organophosphorus compounds can influence their toxicity. Such impurities must be carefully monitored in the technical material and the formulations at the time of manufacturing and after storage in adverse conditions. In 1976, during malaria control operations with malathion water dispersible powder in Pakistan, cases of poisoning occurred including five deaths. The collaborative work carried out after this incident showed that the major toxic component of the malathion wdp was isomalathion. This impurity can potentiate the toxicity of malathion in mammals by inhibiting the normal detoxification mechanism. The Expert Committee on Chemistry and Specifications of Pesticides (WHO, 1978) established new specifications (WHO, 1979a) for malathion and malathion wdp which imposed strict limits on isomalathion. However, vigilance is required for other insecticides which will come out of patent in the near future. These compounds will be produced and/or formulated by various manufacturers with no assurance that during the manufacturing process or due to inert ingredients including in the formulation toxic impurities will not be formed. The World Health Organization prepared and distributed a protocol for simple bioassays of mammalian toxicity of formulations (Vandekar, 1979).

To minimize the possibility of other poisoning incidents and to gain a better perspective of the potential for improving the safety of pesticides, the Expert Committee on Safe Use of Pesticides in 1978 made the following recommendations (WHO, 1979b):
(1) Additional research should be conducted on the toxicity of formulated pesticides after subjecting them to accelerated storage or long-term tropical storage conditions.

(2) The toxicity of pure organophosphorus compounds should be determined for comparison with the commercial materials.

(3) Organophosphorus insecticides containing carboxyl ester moieties should be examined for the presence of impurities.

(4) The toxicology and enzymology of isomalathion and trimethyl and triethyl phosphorothioates should be studied in an attempt to develop an enzymic test for undesirable impurities in field samples of malathion and other insecticides. The new spectrophotometric field kit may be useful for this purpose.

(5) In view of the demonstration in man of the effect of one chemical on the toxicity of another, WHO should pay particular attention to other possible instances of enhanced toxicity of pesticides due to exposure to chemicals.

All these recommendations are being followed up. The concentration of pesticide required for insect control should not be hazardous to the operators. To allow for variations in the performance of spray operators and for difficult field conditions, a relatively high safety factor is required. The safety of an insecticide is partly related to strict observance of precautionary measures during handling and spraying of the formulation. The Expert Committee on Safe Use made the following recommendations:

(1) Mixers and baggers. Since they come in contact with technical materials and concentrated formulations they should wear rubber boots, gloves, aprons and masks. They should be given an adequate supply of soap. All clothing should be washed after each use.

(2) Spraymen. Those applying residual insecticides should be provided with canvas shoes or boots, overalls, and caps with downturned brims; they should be given an adequate supply of soap, and all their clothing should be washed after each use.

A medical examination is advisable, including the determination of blood cholinesterase, for those applying some organophosphorus compounds. For this purpose a field cholinesterase kit has been used for many years, and a spectrophotometric kit for measuring cholinesterase activity has been developed (PDS, 1978b).

Spraymen, mixers, and baggers should be instructed to report any symptoms of illness promptly to the supervisor. The supervisor is responsible for finding out any cases of illness and providing first aid.

Although the operators are generally aware of the danger of inhalation and ingestion of pesticides, they are not always aware that dangerous amounts of pesticides may be absorbed through the skin. Therefore the training of spraymen and supervisors in the safe use of pesticides is essential. The development of new formulations and new modes of distribution may also increase the safe use of insecticides. For example bendiocarb, a rather toxic carbamate, has been safely tested at village scale level after the bagging of the compound at the factory: this reduced the direct handling of insecticide to a minimum.
Larvicides are not as widely used as adulticides in antimalaria programmes. However larvicides must be chosen after careful examination of the possible contacts between man, domestic and wild animals and the treated water. In addition to basic precautionary measures, the safe handling of larvicides may be improved by the use of formulations such as granules, microcapsules or slow release systems. During larviciding operations, the effect on the aquatic non-target fauna must also be monitored.

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4. EPIDEMIOLOGY

4.1 Methodology for evaluation and planning

The use of mathematical models in the epidemiology and control of malaria was further studied during the last five years. An excellent review of the subject was made in 1976.1 A simulation model for the transmission of malaria, which includes the human as well as the entomological factors involved and takes into account the natural resistance to the infection and its uneven distribution, was described.2 The malaria model previously fitted to one-year baseline data from the Garki district in the Sudan savannah of northern Nigeria, was tested against data collected in the same area over a period of three years as well as against data collected in Kisumu, Kenya, also over a period of three years. The test consisted in using the vectorial capacity, calculated from the entomological observations made in the above places and periods, as input in the Garki model, while keeping the other parameters as fitted to the Garki baseline data, and in comparing the prevalence of Plasmodium falciparum parasitaemia as estimated by the model to that actually observed. There was relatively good agreement and the model is considered epidemiologically satisfactory.3

Fenitrothion was evaluated for residual spraying in antimalaria programmes in a large field trial in a hyper-holoendemic area near Kisumu, Kenya, from 1972 to 1976. Data collected on a longitudinal basis indicated that sustained spray protection would reduce malaria prevalence to an asymptotic limit of 6.9%, under the assumption that the inoculation and recovery rates remain stable.4 The effect of fenitrothion was also assessed by measuring the dynamic changes in transmission and on the modifications observed in the infant mortality rates.5 In another study, the insecticidal (propoxur) impact on the mosquito's vectorial capacity, in uniformly and non-uniformly exposed mosquito populations, was assessed from data on the man-biting and age-composition of mosquitos.6

The impact of propoxur on Anopheles gambiae s.l. and some other anopheline populations, and its relationship with some pre-spraying variables, have been also studied.7

A method was described of estimating the malaria incidence rate $\hat{\mu}$ and the recovery $\hat{\rho}$ from longitudinal data. When applied to data on falciparum malaria from the West African savannah, the findings suggested that immunity increases the rate of recovery from patent parasitaemia by a factor of up to 10, and also reduces the number of episodes of patent parasitaemia from one inoculation. Under the effect of propoxur, $\hat{\mu}$ varies with the estimated man-biting rate of the vector while $\hat{\rho}$ increases, possibly owing to reduced superinfection.8

The transmission dynamics of malaria, with reference to P. vivax infections, were studied on the basis of a sensitive mathematical model which was developed for that purpose.9

A 14-month longitudinal survey, integrated with a simultaneously performed entomological study, provided a complete picture of the P. falciparum endemicity in western Ethiopian low lands.10

The concept of risk in parasitic diseases with special reference to malaria, its components and their relationships were described, with emphasis being placed on the value of the parameters to be taken into account in the selection of intervention measures.11

An. labranchiae atroparvus and An. labranchiae were challenged in the laboratory with P. falciparum strains of tropical origin. The results of these experiments suggested that the two mosquitos were not susceptible to infection with the exotic P. falciparum strains.12 On the contrary, An. freeborni, An. maculatus, An. balabacensis balabacensis and five strains of An. albimanus were all susceptible to the Santa Lucia (El Salvador) P. falciparum strain.13
An approach for determining the warning threshold of a malaria epidemic has been proposed. This is based on a two-stage monitoring system, the first level consisting of a warning system based on the surveillance of the relevant indirect factors triggering at appropriate time the second stage monitoring of the epidemiological direct factors closely related to the entomological inoculation rate.14

The recent changes in the epidemiology of malaria in relation to human ecology have been reviewed,15,16

4.2 **Imported malaria**

With the resurgence of malaria in many tropical and subtropical countries during the last decade, the problem of the importation of the disease into freed countries continued to receive utmost attention. WHO publishes every year a list of countries reporting induced and imported cases by country of origin and by species of *Plasmodium*.1,2,3,4,5,6 Russian authors have underlined the problem of malaria importation to the USSR from abroad in a series of articles.7,8,9,10,11,12,13 The importance of the phenomenon was also recognized in other countries, such as France,14,15,16 Federal Republic of Germany,17,18,19 United Kingdom,20,21,22 Portugal,23 Switzerland,24 Czechoslovakia,25 Poland,26,27,28 Canada,29,30 and Japan.31,32 Another outbreak of introduced malaria in the United States of America, the eleventh since 1952, was reported from the Sacramento Valley of California.33

Records of imported chloroquine-resistant *P. falciparum* strains, came from Bangladesh,34 Netherlands,35 United States of America36 and Denmark.37 The last two articles are the first reports of *P. falciparum* chloroquine resistance from Africa.

4.3 **Induced malaria**

The problem of malaria accidentally transmitted by blood transfusion was thoroughly studied in France. A global review was made on the incidence occurring during a 10-year period1 and an inquiry was also made on post-transfusional malaria in France between 1960 and 1974.2 The screening of donors by the indirect immunofluorescence antibody test was recommended as the most efficient prophylactic measure3,4 together with clinical selection criteria5 and possibly with serodiagnosis of malaria being done on homologous antigens.6 The difficulty to demonstrate malaria infection in non-endemic zones following blood transfusion, was thoroughly discussed and actions to be taken for the confirmation of a tentative diagnosis were suggested.7

Cases of transfusion-induced malaria were reported from the United States of America,8,9,10,11 Israel,12 Sri Lanka13 and Mexico.14 There were new reports of induced malaria among drug addicts.15,16 Induced malaria has also been reported among transplant recipients17 and a well-documented case of *P. falciparum* malaria transmitted by platelet concentrate transfusion has been described.18

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4.3 Induced malaria


5. DEVELOPMENT OF TOOLS AND METHODS FOR MALARIA CONTROL

5.1 Insecticides and larvicides

Insecticides remain the most important tools for malaria control.

The World Health Organization has now operated a programme for evaluating and testing new insecticides for 20 years. Up to mid 1977, 1737 compounds were entered in this programme and were evaluated for their effectiveness against disease vectors and for their safety to mammals (document VBC/77.2). Since that time, eight new compounds have been received from manufacturers. The number of new compounds entering annually into the programme reached a maximum in 1962 and then progressively decreased. In 1978, no compounds were offered by the industry. In 1979, six new compounds were received and are being tested. Although new insecticides are now more fully tested by industry before being proposed to the evaluation programme, the situation is still a matter of concern since it is clear that the number of alternatives under development for the future is very low.
As a consequence, it has become uneconomic to maintain field research units for the evaluation of new insecticides at later stages of the evaluation scheme (i.e. stage VI: operational field trials; stage VII: large-scale epidemiological trials). For that reason but also because of reallocation of resources and reorientation of the work of the Organization, away from central technical services and towards national cooperation in the provision of basic health services, some modifications in the evaluation programme have been introduced. The first four stages (i.e. stage I: initial screening, when not already done by the manufacturers; stages II and III: laboratory and simulated field tests and toxicological tests; stage IV: small-scale field tests in experimental houses and ponds) are being maintained as in the past). Stage V (village scale trial) will be carried out by the only field research unit which has not been reoriented and which can test two to three formulations per year under Indonesian conditions. For the further evaluation of new compounds which is still considered essential, it is now suggested that field trials be undertaken by industry in collaboration with national authorities.

A number of insecticides are already operational in malaria control programmes. DDT, HCH, malathion, fenitrothion, fenthion, propoxur. Here emphasis is given to the alternative insecticides newly developed or in development.

5.1.1 Mosquito adulticides

(1) Recommended for testing beyond stage V

- OMS-1197 - chlorphoxim (Bayer), O.P., mamm. toxic, acute oral-hens LD$_{50}$ (mg/kg) 1000 recommended for stage VI. The compound may be safely used in intra-domiciliary residual spraying operations. Highly effective for three months after each application. OMS-1197 had the same impact on the vectors as the standard OMS-43 (WHO/VBC/77.661) on An. gambiae and An. funestus. In Indonesia applied at 2 g/m$^2$, the 50% wdp was effective in reducing the overall population of An. aconitus resistant to DDT for at least 24 weeks. There were no indications of intoxication in either inhabitants or spraymen (WHO/VBC/79.724).

- OMS-1424 - pirimiphos-methyl (ICI Plant Protection), O.P., mamm. toxic, acute oral-rats LD$_{50}$ (mg/kg) 1415. Gave satisfactory control of An. gambiae and An. funestus for three months after each spraying in the Guinea savanna area of Nigeria. Safe for intra-domiciliary residual spraying operations provided the normal precautions are observed (WHO/VBC/77.671).

- OMS-1821 - permethrin (Wellcome), pyrethroid, mamm. toxic, acute oral-rats LD$_{50}$ (mg/kg) 4000. At 0.5 g/m$^2$ gave satisfactory impact on An. gambiae and An. funestus two weeks after each of the two rounds. Safe for indoor residual spraying (WHO/VBC/78.689).

- OMS-1998 - decamethrin (Roussel Uclaf), pyrethroid, mamm. toxic, acute oral-rats LD$_{50}$ (mg/kg) 55. At 0.05 g/m$^2$ gave satisfactory impact on An. gambiae and An. funestus 12 weeks after each of the two rounds. Safe for indoor residual spraying (WHO/VBC/78.689). Extended stage V (group of villages) confirmed 1978 results and showed a higher impact of OMS-1998 on An. gambiae and An. funestus densities due presumably to the protection of the evaluation village from infiltration of mosquitos from nearby unsprayed villages (WHO/VBC/79.712).
5.1.2 Mosquito larvicides

- OMS-786 - temephos (Cyanamid), O.P., mamm. toxic, acute oral-rats LD$_{50}$ (mg/kg) = 1600. At 1 ppm, OMS-786 eliminated all Ae. aegypti adult production for four to six weeks (WHO/VBC/78.594). In Malaysia the effect of OMS-786 E.C. applied at 0.09 kg/ha on An. aconitus lasted two to four weeks. The effect of sand granules at 0.5 kg/ha lasted for more than four weeks. Treatments with OMS-786 are about nine times cheaper than oil treatments. OMS-786 E.C. is now used operationally in Malaysia (WHO/VBC/79.723).

- OMS-971 - chlorpyrifos (Dow Chem.), O.P., mamm. toxic, acute oral-rats LD$_{50}$ (mg/kg) = 200. At 0.1-1.0 ppm effective on Culex quinquefasciatus for 14-24 weeks (WHO/VBC/70.187), (WHO/VBC/73.452), (OCCGE/296/ENT/69), (OCCGE/41/ENT/69). At 1.0 ppm effective on Aedes aegypti for 12-17 weeks (WHO/VBC/74.474) (OCCGE/19/ENT/74).

- OMS-1211 - jodfenphos (Ciba-Geigy), O.P., mamm. toxic, acute oral-rats LD$_{50}$ (mg/kg) = 1600. At 1.0-5.0 ppm effective on C. quinquefasciatus seven to 25 days (WHO/VBC/70.187) At 0.5-1.0 ppm effective on Aedes aegypti seven to 16 weeks (WHO/VBC/74.474) (OCCGE/19/ENT/74).

- OMS-1424 - pirimiphos-methyl (ICI Plant Protection), O.P., mamm. toxic, acute oral-rats LD$_{50}$ (mg/kg) = 1415. At 0.5-1.0 ppm effective on Ae. aegypti seven to 11 weeks (WHO/VBC/74.474) (OCCGE/19/ENT/74).

- OMS-1697 - methoprene (Zocon), IGR. At 1.0 ppm gave almost total inhibition of C. quinquefasciatus adult emergence for 21 days, 80% inhibition for 30 days. At 10.0 ppm 70% inhibition for 60 days (WHO/VBC/76.604).

- OMS-1804 - diflubenzuron (Philips Duphar). At 1.0 ppm gave 100% C. quinquefasciatus adult control for 32 days. At 2.0 ppm gave 100% Ae. aegypti adult control for 42 days (WHO/VBC/77.655).
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5.2 Environmental management measures

The application of environmental management measures for mosquito control was a time-honoured technique. This technique was all but neglected since the introduction of residual insecticides, the use of which produced dramatic effective results on the control of mosquito vectors at low costs. However, limitations of chemical control have become obvious in recent years. The need for incorporating environmental management methods into integrated strategies for malaria control is therefore increasingly felt.

One of the activities carried out by WHO in the promotion of environmental management measures for vector control was the seminar on the Prevention and Control of Vector-Borne Diseases in Water Resources Development Projects, held in Egypt and the Sudan in March/April 1978. A major undertaking of the seminar was a general review of the health implications of water resources development projects and of the environmental management methods that can be applied for the prevention and alleviation of these problems, primarily concerning malaria and schistosomiasis. The report of the seminar (document VBC/EM/78.1) has been well received and provides useful guidelines to the vector control workers as well as to the water resources development engineers. It may be noted that as a result of the seminar, the Blue Nile Health Project in the Sudan, which follows a comprehensive approach for the prevention and control of water-related diseases, has been formulated and is now in operation.

An Expert Committee on Environmental Management for Vector Control was convened in Geneva in November 1979. Its report, which contains the experts' views and recommendations in this field and provides authoritative guidance, is in an advanced stage of publication in the WHO Technical Report Series.

For environmental management to be successful in the control of vector-borne diseases, close collaboration between national, international and bilateral agencies concerned with water development, agriculture and the environment is essential. One of the recommendations of the Expert Committee was to establish and maintain such collaboration in particular at the international level. A Memorandum of Understanding between WHO and FAO regarding collaboration in the prevention and control of water-borne and associated diseases in agricultural water development activities was signed by the two Directors-General in January 1978. As an expansion of that Memorandum of Understanding and in pursuance to a recommendation of the above-mentioned Expert Committee, a WHO/FAO/UNEP Task Force on Environmental Management for Vector Control is being established, to take the responsibility of following up the implementation of the Expert Committee's recommendations, in addition to ensuring interagency collaboration.

A Manual on Environmental Management for Mosquito Control with a special emphasis on malaria vectors has been compiled and its first manuscript is being circulated to selected reviewers for comment. The Manual includes treatise on environmental modification, environmental manipulation, reduction of man/vector contact, as well as the planning of environmental management activities for mosquito control under different situations. It must be reiterated here that environmental management measures were used for malaria control early in the century and the immediate action now required is to re-disseminate the knowledge and experience accumulated during that period with recommendations for their application in present day vector (or disease) control programmes and land and water development projects. Technical or scientific advancement in the form of improved technology can be expected in a few years through pilot studies, field trials and extended applications of these measures in a well-planned and cost effective manner in field projects such as the Blue Nile Health Project in the Sudan.

On the operational side, a consultant visited the Solomon Islands in 1979 to advise on the control of mosquito breeding in coastal lagoons by means of environmental management methods. The recommendations contained in the consultant's report (published by WPRO as an Assignment Report dated 20 August 1979, ref. ICP MPD 002, SOL MPD 001) should have application
in similar situations in many coastal areas of Indonesia and other countries in that region. Also, the actual implementation of these recommendations would test their applicability value and feasibility and may lead to further improvement in the recommended technology for greater effectiveness and economy in subsequent applications.

A list of papers prepared by EPO for the years 1975-1979 with reference to environmental management for mosquito control follows.

**LIST OF PAPERS PREPARED BY EPO (1975-1979) REGARDING ENVIRONMENTAL MANAGEMENT FOR MOSQUITO CONTROL**

Endemic diseases in agricultural development and food production.

The current situation and prospects on pesticide supply and demand in public health programmes.

Investment required in pesticides and equipment supply, and mechanism for their reserve and emergency operations.

Role du desherbage dans les programmes de lutte contre les maladies parasitaires.

Lutte contre les maladies parasitaires autres que le paludisme problèmes actuels et perspectives d'avenir.

Planification de la reduction des sources dans les programmes de lutte contre le paludisme et d'autres maladies parasitaires. (With draft in English.)

L'action physique dans la lutte contre la paludisme et les autres maladies parasitaires

Pesticides availability and costs (prepared for the Twenty-seventh World Health Assembly Report of the Director-General, Development of the Antimalaria Programme) (Annex II to A28).

Vector control policy to counter resistance in specific programmes of control of vector-borne diseases. (Prepared for Expert Committee on Resistance of Vectors and Reservoirs to Pesticides - September 1975.)

Recent developments in antimalaria operations. Progrès recents en matière d'opérations anti-paludiques.

Technical and general justifications for conducting antilarval operations in malaria control programmes.
Comparative effectiveness and cost of various antimalaria measures directed against malaria vectors under different situations. (Prepared in collaboration with Dr M. A. Farid.)

The present status of the control of schistosomiasis.

Etat d'avancement de la lutte contre la schistosomiasse.

The present status of the control of filariasis.

Situation actuelle de la lutte contre la filariose.

The present status of the control and eradication of malaria.

An overall review of the WHO programme of mosquito control (antimalaria projects).

Total insecticidal coverage. (19.11.59)

The impact of irrigation and drainage on public health.

Projects for control of parasitic diseases. (Programme for the control of endemic diseases - 1st draft.)

Control of endemic parasitic diseases (2nd draft). Lutte contre les maladies parasitaires.

Parasitic diseases and human settlements.

DDT availability and costs.

A review of problems and progress in vector control operations of parasitic diseases control and eradication programmes.

The increasing cost of pesticides as an impediment to vector control operations.

Notes on the feasibility and economic aspects of control.

Enquêtes de faisabilité et aspects socio-économiques des programmes de lutte contre les maladies parasitaires.
A short summary of WHO in the use of antimosquito measures and integrated control for antimalaria programmes.

Methodology of malaria operations.

DDT and replacement residual insecticides for antimalaria operations.

A comprehensive approach to vector control in malaria and other parasitic diseases' control programmes.

Une approche globale de la lutte antivectorielle dans les programmes de lutte contre le paludisme et d'autres maladies parasitaires.

Chemical methods of malaria control (insecticides) (paper in English, French and Spanish).

Environmental Management for Malaria Control (paper in English, French and Spanish).

The use of larvivorous fish in antimalaria programmes (paper in English, French and Spanish).

Development, testing and introduction of environmentally sound methods of malaria control (paper in English, French and Spanish).

A systems approach to antimalaria programmes (paper in English, French and Spanish).

Training needs (paper in English, French and Spanish).

The place of pesticides in public health programmes (for Bangkok UNIDO Symposium 1-7 February 1977)

Projets hydro-agricoles et maladies transmissibles

Pesticides in Antimalaria Programmes - A global summary

Criteria for the selection of malaria control methods

Assessment of malaria control programmes (operational)
Biological control

Biological control of vectors is receiving increased attention as it could become an important component of integrated vector control strategies and provide new tools for controlling vectors resistant to conventional insecticides. Furthermore, there is a distinct possibility that certain biological control agents could be produced by communities as part of their self-protection against some vector-borne diseases.

Research on the biological control of vectors was originally limited to the screening, evaluation and development of parasites and pathogens. Now it also includes parasitoids and predators, in particular fish, as well as competitors which could displace the vector or reduce its abundance to below epidemiologically critical levels.

Pathogens and predators

The most progress has been made with one spore-forming bacteria serotype H-14 of Bacillus thuringiensis, and one nematode Romanomermis culicivorax, both of which are reaching the operational trial stage. Great hopes are also placed on another spore forming bacteria, the strain 1593 of B. sphaericus, the nematode R. iyengari, a series of fungi (Culcinomyces, Coelomomyces, Lagenidium, Leptoleginia, and Metarhizium) and the predatory mosquitos Toxorhynchites.
Mosquito larvivorous fish

A number of species of fish have been used in the past for the control of mosquito larvae, with special emphasis on those of malaria vectors. Gambusia has been employed in North America since the turn of the century and by the 1930s it had been introduced throughout the world. The epidemiological effectiveness of the fish has been very rarely documented and the only clear-cut success reported in literature deals with the massive use of Gambusia in areas of unstable or hypoendemic malaria. Although the advantage of synthetic insecticides resulted in a sharp decrease of interest in the use of predatory fish, during recent years an increasing interest for the use of larvivorous fish has emerged due to their importance in situations where vector resistance to insecticides is found.

WHO, therefore, initiated and stimulated further interest in the use of larvivorous fish for the control of malaria. During 1974, a questionnaire on the use of larvivorous fish for the control of mosquito larvae was sent to all the Member States of WHO. The detailed information in respect of each country has been tabulated. Out of the 12 different kinds of fish, the species most commonly used were Gambusia, followed by Tilapia and Poecilia.

With regard to the efficacy of fish for the control of mosquitoes the impact has been determined mostly on an empirical basis. In some countries remarkable results have been reported, while in others the results were rather disappointing.

WHO also organized a travelling seminar on the use of larvivorous fish for mosquito control in anti-malaria campaigns in Bulgaria and the USSR during 1979. The purpose of the travelling seminar was to discuss and expose the senior vector control and malaria operations officers to the potential advantages and disadvantages of this method of vector control.

A few specific instances of successful use of fish for anopheline control may be cited.

In Korea, a series of laboratory and field observations on two species of local fish, Aplocheilus latipes and Zacco platypus for the control of the vectors of malaria, An. sinensis breeding in rice fields, marshes, swamps and ditches where these species of fish are also found were carried out and Z. platypus gave more promising results. This species of fish survives under a variety of environmental conditions, including a wide range of temperature (0-40°C) and has a voracious appetite for mosquito larvae. In a simulated rice paddy field successful control of Anopheles and Culex larvae was obtained for a month after the introduction of these fish at the rate of a pair per m² of water surface.

In the Maldives Republic, larvivorous fish Poecilia reticulata (=Lebistes, guppy) and another two species of fish which have a high tolerance for brackish water and are well adapted to well environment have been studied. Anophelines incriminated as malaria vectors are An. tessellatus which exclusively breeds in wells and An. subpictus which breeds in brackish water or marshes and the effectiveness of the fish in controlling mosquito larvae is drawn only from circumstantial evidence.

An early review of the literature revealed that 216 fish species from 30 families had been used against 35 mosquito species in 41 countries to control the yellow fever mosquito (Aedes aegypti) and the malaria mosquito (Anopheles spp.).
In Pondicherry, India, laboratory observations and a field trial to assess the potential of several larval fishes (Oryzias melastigma, Aplocheilus blochii and Poecilia reticulata) as well as the previously introduced G. affinis in controlling mosquito breeding in wells, such as An. stephensi and An. subpictus have been carried out.

It was concluded that Aplocheilus blochii and Oryzias melastigma are more effective than G. affinis. Both species are widely distributed in India. Another species which has been used quite extensively and shows some promise is Poecilia reticulata. In Indonesia it has been used quite successfully. A study is in progress to obtain information on the biology and efficiency of this fish. It can thrive well under various environmental conditions, particularly in polluted waters.

In Somalia, Nothobranchius already exists in the southern region. Tilapia has also been seeded. A large-scale trial on Tilapia zillii in man-made reservoirs in the semi-desert area has been planned for 1980 under the auspices of the Special Programme for Research and Training in Tropical Diseases.

The Fisheries Research Centre in Gezira is experimenting with two herbivorous fish, namely the Chinese Grass Carp (White-Armur) and the Common Carp for weed control in the canals.

Under the aegis of the Special Programme for Research and Training in Tropical Diseases, the Scientific Working Group on Biological Control of Insect Vectors of Diseases recently made a brief review of some current studies to acquire a broader understanding of prospects for future use of fish as biological agents for vector control and established the following criteria:

(a) efficiency of fish in control of mosquito larvae;
(b) tolerance of fish to environmental conditions;
(c) potential for mass production;
(d) tolerance to insecticides;
(e) ecological impact of introduced fish.

Based on these criteria, Zacco platypus and Poecilia reticulata were considered to have a great potential.

Other fish species, including the common grass carp S. idella, A. dispar, Nothobranchius and Tilapia sp. also have potential, but at present no specific studies are in progress and little is known of their biology. The SWG stressed that the full impact of the introduction of fish as an environmental contaminant factor in local ecological conditions has not been fully investigated. Gambusia species have not been recommended because of their likely impact on non-target fauna.
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5.4 Genetic control

Genetic control may aim at either direct suppression of a vector population or substitution of an existing population with a modified one which will not transmit disease or is more amenable to control by other means. In population suppression it may be more realistic to attempt to reduce the vector density below a critical level so that disease transmission ceases rather than to eradicate the vector which may be possible only under special situations.

Sterile Insect Release Method (SIRM) has been most successful in population suppression. A population of An. albimanus was reduced by 99% in an isolated lake area in El Salvador in 1972 by release of chemosterilized males. However, irradiated or chemosterilized males may lack mating competitiveness. Genetic systems that confer natural sterility are hybrid sterility, cytoplasmic incompatibility and chromosomal translocations. Crosses between the 6 species of the An. gambiae complex produce sterile males with atrophied testes devoid of spermatozoa. A field trial in Upper Volta, however, did not succeed due mainly to possible mating barriers between the males of a cross between An. arabiensis and An. melas and the local target species, An. gambiae s.s. It may therefore be necessary to arrange that the release insects have the same genome as the target population and differ only in the sterility factor. In An. gambiae, stocks have been produced with the X and Y chromosomes of Species B and the autosomes partly from Species A. This stock would produce sterile male progeny when mating with Species A and may be expected to show effective mating competitiveness in the field. In the case of cytoplasmic incompatibility (extensively studied in Culex quinquefasciatus) it has been found easier by crossing to introduce the genome of a Delhi strain of the species into Paris cytoplasm and have the resulting stock compete well for mating after release in the Delhi area. Chromosomal translocations cause meiotic abnormalities and thus lead to varying degrees of sterility. The translocation heterozygotes show hybrid vigour and transmit sterility to the subsequent generations until natural selection may eventually eliminate it. The effect can be enhanced by combining a meiotic drive factor causing a preponderance of males.
The first successful SIRM control of a pest (screw worm) was accomplished without a genetic basis. However, studies are needed to gauge the genetic heterogeneity of the target population and also to ensure quality control of the mass-reared males so that they can successfully mate with the target females. Genetic methodology also makes it possible to achieve enhanced competitiveness of the released insects through deliberate selection of favourable traits or providing for hybrid vigour through hybridization before release. Mechanical separation of the males for release is usually applicable at an advanced stage of the life cycle only (pupae) and may not be sufficiently effective (86% efficiency for An. albimanus). Genetic sexing however, allows the selective killing of females in the egg or larval stage and thereby effects an enormous saving in rearing costs (over $4,000 per million An. albimanus males in a project in El Salvador). Genetic sexing systems are now available for An. albimanus, An. gambiae s.s. and An. arabiensis.

Population suppression or eradication may be followed by occupation of the ecological vacuum by immigrants of the original or other species. Such a possibility may be avoided in population replacement. Refractory strains have been developed in the laboratory and include An. stephensi to Plasmodium gallinaceum, An. gambiae to P. berghei and P. yoelii and An. arabiensis to P. berghei. A gene conferring susceptibility to a cheap and effective insecticide like DDT could be introduced into a population by release of fertile males. Releasing large numbers of males and a few females of a strain carrying a desirable gene into a population of a strain with which it would have sterile matings or produce sterile progeny would result in the ultimate replacement of the latter strain by the former. The combination of natural sterility factors and transporting mechanisms such as meiotic drive, compound chromosomes etc. would permit reduction of the number of releases and numbers released with longer term benefits.

In conclusion, while there is a choice of genetic control methods and much work is being currently done on important malaria vectors such as An. albimanus, An. gambiae species A and B, An. culicifacies and An. stephensi, it is safe to assume that much more work needs to be done before any one method may reach an operational stage is recognized by the most recent WHO Expert Committee on Malaria.

REFERENCES


