use of the tools available for control as well as the development of new tools and methods which would allow the reduction and ultimately the elimination of malaria. Moreover, the cost of these tools and methods should be within the reach of the countries exposed to the disease. The development of such technologies can only be expected from research in the laboratory and in the field related to chemotherapy, immunology, epidemiology, and control or from supportive studies on parasite biology. In this context, it would be an error to give absolute priority to one research discipline and to neglect the others since past experience has shown that reliance on one or two selected control methods is insufficient to bring about a lasting reduction in or the elimination of parasite reservoirs in areas with stable malaria.

During the last decade, there has been a renaissance in malaria research directed towards these objectives. Government agencies and services in several countries, and international organizations such as the European Community and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases have made available funds which have provided an opportunity for expanding and accelerating the research effort. The main objectives of these programmes are to make better use of old drugs, to develop new drugs, vaccines, and immunodiagnostic tests, to undertake biological studies which may provide leads or assist the development of new tools for malaria control, and to improve available methods and their application for control of the disease in the field with particular emphasis on the epidemiology of the disease. It is also encouraging to note the involvement of an increasing, albeit small, number of pharmaceutical industries without whose participation the laboratory advances could not be translated into usable and practical tools. The present time is therefore an exciting one for malaria research, some of the advances of which are highlighted below.

The biology of the parasites

The development of techniques for the cultivation of the erythrocytic stages of P. falciparum by Trager and Jensen (6) had a tremendous impact on malaria research in the mid 1970's. Their methods made it possible for research workers throughout the world to study this clinically most important malaria parasite, forming the basis of many recent advances in malaria biochemistry, parasitology, immunology and chemotherapy. As a consequence, drug-screening methods were developed (7), and important studies were carried out on the invasion of the red cell by the parasite and on subsequent changes in the infected cell as the parasite developed. These latter studies have not only proved to be important for a greater understanding of host parasite relationships but have also had a major impact on antimalarial vaccine development. The more recent development of techniques for the in vitro cultivation of the exoerythrocytic stages of malaria parasites, first for P. berghei (8) and later for P. vivax (9), has already had an impact on immunological research on these stages (10) and these techniques may prove to be equally applicable to screening for tissue schizontocides. However, in order to detect antirelapse activity, it still has to be shown that hypnozoites develop in the cultures of P. vivax. In any event, these techniques may prove to be as important a tool in the future as the development of techniques for the cultivation of the erythrocytic stages of P. falciparum did in the late 1970's.

Culture techniques have led during the last ten years to a greater understanding of the method of invasion of host cells by the parasite. It has been postulated for many years that the interaction of the merozoite and the red cell depended on specific receptors on each cell but it is only recently that putative receptors have been identified. On the basis of studies with red cells specific for various blood group antigens and of inhibition studies with various erythrocytic components, it has been shown that glycophorins A and B on the red
cell surface are involved in the recognition of merozoites of *P. falciparum* (11,12). The initial interaction is thought to be dependant on the carbohydrate moieties of glycophorin, possibly related to the presence of one or both sugars of the disaccharide structure N-acetyl neuraminic acid-galactose (NeuNAc 2-1 Gal) followed by a higher affinity interaction involving the hydrophobic domain, i.e. T₆ fragment, of glycophorin A (12). In addition, potential receptors for attachment and invasion have also been identified on *P. falciparum* merozoites but their role has yet to be confirmed. A doublet of *P. falciparum* proteins, Mr 155 000 and 130 000, localized on the merozoite surfaces have been shown to bind to glycophorin A and B (13). Monoclonal antibodies to these proteins also block invasion *in vitro* thereby implicating these molecules as functional receptor components. In addition, a glycoprotein of higher molecular weight ranging between Mr 190 000 and 230 000, has been identified on the surface of merozoites and schizonts of a variety of malaria parasites (14). This protein which, in *P. falciparum*, has an Mr of 195 000 appears to vary antigenically within the species and contains species- and strain-specific epitopes. It is apparently processed at the time of merozoite release from infected red cells to lower molecular weight components. Its role in invasion is unknown but it appears to be involved in the host's immune response to the disease. Proteins, associated with the paired rhoptry organelles at the merozoite apex, have also been identified by monoclonal antibodies. These appear to be divided into two distinct classes with Mr ranges of 40 000-90 000 and 100 000-150 000 (14,15). Monoclonal antibodies to these proteins block invasion of *P. falciparum*, thereby indicating the potential of such proteins as antigens for vaccination purposes. It has been suggested that some of the proteins may be involved in the process of invasion leading to vacuole formation.

Until recently nothing was known of the mechanism of entry of malaria sporozoites into the host cell. This was mainly due to the lack of an appropriate test system. However, this constraint has been overcome by the development of the techniques for the *in vitro* cultivation of the exoerythrocytic stages referred to above. By using these techniques it has been shown that sporozoites adhere to the surface of the largest cell and enter it following the formation of a parasitophorous vacuole by the apparent invagination of the target cell membrane. This invasion process including the attachment phase may be inhibited by monoclonal antibodies to the circumsporozoite protein or the corresponding Fab fragments at concentrations which neutralize sporozoite infectivity (10,16). These results and others which indicate that these sporozoite specific antigens are probably associated with the target cell membrane suggest that the sporozoite surface protein is intimately involved in the entry of sporozoites into cells. Moreover immunity to sporozoites appears to be mediated by the binding of antibody to the parasite surface thus inhibiting its interaction with the target cell. This appears to be the mode of action of the potential sporozoite vaccine for malaria.

Malaria vaccines

These advances and others in the field of immunology have been responsible for the optimism of the scientific community about the feasibility of developing effective vaccines against the disease. With the application of monoclonal antibody technology and DNA cloning techniques to malaria as well as the expectation that new technologies for vaccine production will also be applicable to malaria, such optimism may be justified. However, the complex structure and life cycle of the parasite and the steps required to the develop a vaccine to the point that it can be tested in man preclude any quick solution to the successful development of a vaccine which can be used in the field.

Malaria parasites undergo a complex cycle of development, part of which takes place in the vertebrate host and part in the mosquito vector. Both
morphological and antigenic changes occur during this cycle. As a consequence, the parasite confronts the host with a large number of antigenic components, many of which will be distinct and stage-specific but of these only a small proportion are likely to stimulate protective immune responses. The rest are either irrelevant to protection or may induce a variety of undesirable or futile host responses. Thus the use of whole parasites for vaccination purposes is not a feasible approach, neither is it practical since parasites cannot be produced in sufficient quantities or with a high enough degree of purity for such purposes. The present strategy for malaria vaccine development is therefore based on the identification, characterization and production of those parasite antigens which specifically stimulate protective immune responses. This requires the cloning of the genes coding for the protective antigens and their expression in bacteria, the analysis of their nucleotide sequence and determination of the amino acid sequence of the encoded molecule and the production of the molecule or its epitope(s) by genetic engineering methods or by chemical synthesis.

Three methods of vaccination against malaria are currently being investigated. Their object is either to prevent any infection occurring (a sporozoite vaccine), to alleviate clinical symptoms (asexual blood stage vaccine), or to prevent transmission (a gamete related vaccine). However, as protective immunity in malaria is stage specific, a blood stage vaccine will not prevent infection by sporozoites and a gamete-based vaccine will not prevent infection by sporozoites or the development of disease due to the asexual stage. Dependant on the epidemiological situation and the target group one can envisage the need for future vaccines based on pure antigens of either one or more of these stages.

Considerable progress has been made in the last few years in the identification and characterization of protective antigens from all three stages. The protective antigen from sporozoites which is the major surface membrane protein has been characterized in several species of plasmodia. These circumsporozoite proteins are immunodominant molecules which share a number of characteristics. They have been identified and characterized from P. berghei (Pb44) (17), P. knowlesi (Pk42) (18), P. cynomolgi (Pc48) (19), P. falciparum (Pf58) (20) and P. vivax (Pv45) (20). The letters with figures in brackets indicate the species concerned and the molecular ratio (Mr) of the protein x 10³. Subsequently the production of the protein by recombinant DNA techniques and of the immunodominant epitope by chemical synthesis proved to be feasible. More important, however, has been the cloning of the gene coding for the circumsporozoite protein of P. falciparum. This was achieved independently and almost simultaneously by two different groups of scientists. One group used standard methods to construct a cDNA library from mRNA extracted from sporozoites (21) whilst the other employed mung bean nuclease to excise the gene coding for the sporozoite protein from the genome of the asexual blood stages (22). Both groups carried out sequencing analysis and found that the immunodominant epitope contained repeated sequences of two chains containing 4 amino acids in each. The repeated sequences were synthesized and shown to share common antigenicity with the native circumsporozoite protein.

Asexual blood stage antigens form an extremely complex group. They are present in large numbers, some of which occur in contact with host cell components while others undergo processing during the cell cycle to yield other products of different molecular ratio. Identification and analysis of these proteins is therefore difficult. Of major interest are:

- the antigens exposed to the immune system on the surface of schizonts and merozoites;
- antigens which react with the monoclonal antibodies that inhibit parasite growth in vitro;
- antigens which react preferentially with serum from immune adults from endemic areas and with IgG from sera of immune animals conferring protection on
passive transfer; - antigens which play an essential role in parasite functions such as invasion of the host cell.

Thus, those proteins identified as putative merozoite receptors (i.e. those with Mr's of 155 000 and 130 000 (13,23), the merozoite surface protein of Mr 195 000 and the rhoptery proteins) are all antigens which may be considered for inclusion in an asexual blood stage vaccine against P. falciparum. Another antigen of potential importance is one which has been identified on the surface of red cells infected with late trophozoites and schizonts (24). This antigen is associated with the surface knobs which are the site of attachment of infected erythrocytes to endothelial cells in vitro (25). It is thought that these knobs are involved in the sequestration of erythrocytes containing mature parasites thereby enabling them to disappear from the peripheral circulation and evade destruction in the spleen. This is probably an important pathogenic mechanism in cerebral malaria. Immune serum can prevent or reverse cytoadherence and it has been shown that the knobs are associated with antigens of parasitic origin. Knob production is associated with the synthesis of a histidine rich protein (HRP) of Mr 80-110 000 resembling in amino acid content a similar protein from rhopteries of P. lophurae which induced protective immunity to that parasite in ducks (26). Recently HRP-related genes and transcription products have been identified in P. lophurae. Highly repetitive DNA sequences were found in the coding region of the HRP gene which has now been cloned (27). These are not the only blood stage antigens which may have relevance to vaccine production. Others have been described at this stage in less detail and so their significance has yet to be determined.

Transmission blocking immunity has now been studied in P. gallinaceum, P. yoelii, P. falciparum and P. vivax. The development of techniques for stimulating gametocyte production in in vitro cultures of P. falciparum has allowed sufficient numbers of gametocytes to be produced for the identification of target antigens. Transmission blocking monoclonal antibodies have been shown to react to gamete surface molecules of Mr 260 000, 60 000 and 55 000 which closely resemble the P. gallinaceum antigens identified in earlier studies (14,28). Studies on transmission blocking immunity in P. vivax have been conducted on gametes collected from human patients in the absence of suitable techniques for the cultivation of erythrocytic stages of this parasite in vitro. However, in spite of these difficulties immunization of rabbits with vivax gametocytes produces antisera which abolish infectivity and studies are being conducted to identify the antigens involved in this process.

Thus it may be said that the prospect of developing vaccines against malaria parasites are good. However, all of the major problems have not yet been solved. Although a number of candidate antigens on which vaccine development might be based have been identified, there is still a long way to go before any vaccine might be ready for field trials. For example, the production and formulation of vaccines for use in man may pose problems, e.g., the antigens may require carrier molecules or adjuvants. It remains therefore impossible to predict when a vaccine will become commercially available. In addition, tools will have to be developed for the field evaluation of future malaria vaccines. For this purpose, and for epidemiological studies in general, there is a need for new diagnostic methods for malaria and the improvement of existing ones. The ideal diagnostic test should be sensitive, specific, reproducible, inexpensive, rapid and simple to perform. In addition there is a need for tests able to detect low-grade infections, to differentiate between a current and a past malaria experience, to distinguish primary infections from relapses and recrudescences, and to measure immunological status and protection.

Although the conventional, microscopical blood film examination is a sensitive technique for parasite detection, it has several limitations when used
under field conditions. Besides being time consuming and laborious, it is a subjective test which becomes less reliable for monitoring infections with low parasite densities unless used by highly trained personnel. Large-scale studies, in particular, would be greatly facilitated by simple tests for detecting parasites in the peripheral blood with a high degree of sensitivity. Both radioimmuno assay (RIA) and enzyme-linked immunosorbent assay (ELISA) methods have been devised for the detection of \textit{P. falciparum} in blood and useful levels of sensitivity have been obtained (29, 34). These are solid-phase assays based on competition or inhibition of binding of specific antibody to parasite antigens. These methods can also be used to detect malarial antibody. However, greater precision as well as standardization will be required before such tests are ready for widespread use in field studies. This desired standardization and increased sensitivity should be made possible by the use of standardized reagents such as defined antigens for antibody detection and defined antibodies, (e.g., mono or polyclonal), for parasite detection. A simple solid-phase sandwich technique has already been devised using monoclonal antibodies to the circumsporozoite protein for the detection of sporozoites in infected mosquitos (35). This technique appears to be highly sensitive, quantitative and species specific. Another approach to malaria diagnosis, based on the use of a probe containing repetitive sequences of \textit{P. falciparum} DNA, has been described by Franzen et al. (36) and is described in more detail by Wilairat in this journal (37). This is a promising technique which, in its present form affords a greater sensitivity than that obtained by an average microscopist though not by a good one (38). Further refinements, including the application of non-radioactive methods as well as the development of similar probes for \textit{P. vivax}, \textit{P. malariae} and \textit{P. ovale} are required before such a technique could have a wide epidemiological application.

The assessment of protective immunity in malaria also remains a problem since there is still no test which measures protection. Several methods are available for the measurement of serum antibodies but they do not measure protection or distinguish between past and present infections. Functional assays in which antibody reacts with antigens involved in the development of the parasite have been devised, e.g., the merozoite invasion inhibition assay and a test based on the inhibition by sera of the binding of trophozoite-infected erythrocytes to endothelial or amelanotic melanoma cells, but their correlation with protection has yet to be confirmed in field studies.

Good evidence exists from animal models to show that cell-mediated responses are involved in protective immunity to malaria particularly to the blood stages of the parasite. Tests for measuring such responses are now under development for application in epidemiological studies and for the future evaluation of malaria vaccines.

Chemotherapy of malaria

Any future malaria vaccine(s) may add to the range of measures for controlling malaria but it will not remove the present dependance on other traditional control measures such as the use of antimalarial drugs. Moreover, in view of the emergence and spread of multi-resistant isolates of \textit{P. falciparum}, there is an urgent need to develop new drugs and formulations with novel structures and modes of action. In this context, the registration of the first new antimalarial drug for 30 years occurred in 1984. This was for the quinoline, methanol, mefloquine, which has been registered as the monosubstance and as a triple combination with sulfadoxine and pyrimethamine. These formulations have been developed by Hoffmann-La Roche in collaboration with the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and the Walter Reed Army Institute of Research.

Mefloquine is a potent blood schizontocide active against multiresistant \textit{P. falciparum} malaria (39). It has now been studied clinically as the monosubstance