interestingly, in 1885—and to claims that doctors would obtain cover more cheaply from commercial insurance. *Mishap or Malpractice?* explores these possibilities and dismisses them. Indeed, the BMA's annual representative meeting has twice drawn back from embarking on this course. Most doctors undoubtedly like and respect their defence organisations and have no wish to change to the entrepreneurial and ruthless world of commercial insurance. The union's status of a mutual non-profit-making defence organisation, confirmed in the courts in 1979, is something with which they feel comfortable.

Among the advantages of this "mutual defence" system welcomed by doctors is the facility to ring up a defence body and talk frankly to a doctor about their problems. This means a large medical staff—in its London office alone the MDU has 15 doctors and dentists—backed up with appropriate legal advice. The cost of this, however, is small compared with the major costs of the defence organisations—going to court—and it is one that the profession is undoubtedly willing to pay for. Among the many quests for

advice will be some on patient-doctor confrontation, consent, confidentiality, and no fault compensation, subjects with which all practising doctors should at least be familiar and on which Dr Hawkins writes with clarity. Questions about patients seeing their own records, putting records on the computer, and the Data Protection Act 1984 are acquiring a relevance to daily practice on a level with queries on clinical matters.

The MDU has serviced the profession well under a succession of eminent presidents and secretaries. Dr W D Wylie, the incumbent president, and Dr J W Brooke Barnett, secretary, may be proud that in celebrating the union's centenary they lead a strong and respected organisation. The pace of social and medical change is at times frightening. But for the foreseeable future doctors will be turning with confidence to the MDU for help—preferably to avoid medicine's multiplicity of hazards but otherwise to deal with their consequences.

1 Hawkins C. *Mishap or malpractice?* London: Blackwell, 1985.

Regular Review

Recent trends of chemotherapy and vaccination against malaria: new lamps for old

L J BRUCE-CHWATT

We have three [fellows] that bend themselves looking into experiments of their fellows and cast about how to draw out of them things of use . . . in man's life and the easy and clear discovery of the virtues and parts of bodies. These we call Dowry-men or Benefactors. . . . We have three others that do execute the experiments so directed and report them. These we call Inoculators.

FRANCIS BACON, *New Atlantis*, 1627

Data released by the World Health Organisation in 1985 show that no fewer than 6.5 million cases of malaria were recorded in 1982.¹ These figures represent only a part of the true picture: the data from tropical Africa were not included because of the incomplete and uncertain reporting from the large endemic areas of that continent. Of the total world population (1983) of some 4.7 billion, about 2.2 billion live in places where the incidence of malaria has been virtually eliminated or reduced in varying degrees, but almost 400 million people in rural tropical areas are exposed to its full brunt. No fewer than 373 million inhabitants of sub-Saharan Africa live in endemic areas where *Plasmodium falciparum* is the prevalent species. The annual number of clinical cases of malaria has been variously estimated at between 76 and 150 million cases.

A massive resurgence of the disease in South East Asia seen during 1976-8 has now decreased to 1973 levels, but there has been a slow rise of incidence in south and central America,

while the picture in Africa has shown little change over the past 20 years.¹

In 1969 the World Health Organisation recognised that (despite success at the periphery of the natural distribution of the disease) global eradication of malaria was not attainable for technical, administrative, socioeconomic, political, and other reasons. Its new, more flexible strategy of malaria control put the emphasis on closer collaboration with basic health services in urban and rural areas.² Ten years and 2650 million dollars later the World Health Assembly, responding to the call of the Alma Ata conference of 1978, emphasised the need for malaria control to be integrated with primary health care.³ By 1980 about 51 anopheline species of malaria vectors had become resistant to dicophane (DDT) and other insecticides making it clear that in rural areas of developing countries control measures will need to take more account of all available methods, including the wider use of antimalarial drugs for treatment of the disease and for its prevention.^{2,4,5}

Chemotherapy

The modern history of chemotherapy of malaria has been one of ups and downs.⁶⁻¹⁰ Our present greater than ever dependence on the curative and preventive virtues of anti-

malarial drugs has coincided (paradoxically and rather cruelly) with the spread of resistance of *P falciparum* to chloroquine (fig 1), hitherto our most reliable compound, and to some alternative drugs.⁸⁻¹⁰⁻¹² Resistance of this species to chloroquine is found in small or large foci in at least nine countries in south and central America, 15 countries of Asia and Oceania, and some 12 countries of sub-Saharan Africa. It is more widespread and of a higher degree in South East Asia than anywhere else. The worrying trend is that resistance of *P falciparum* to chloroquine seems to stimulate resistance to other compounds and appears to confer to the resistant strains a greater capacity for transmission.¹⁰

A recent World Health Organisation report, largely concerned with drug resistance in human plasmodia,¹² described the mechanism of its development, its present geographical distribution, methods of measuring the degree of resistance of *P falciparum*, and means of prevention; but the report also points to the need for improved knowledge of the genetics and molecular basis of drug resistance. Much research on new antimalarials has been carried out by the United States Army Medical Research and Development Command and the Walter Reed Army Institute of Research. Its programme has screened well over 250 000 compounds but has produced only a few promising leads. Another collaborative research programme on the chemotherapy of malaria is being pursued jointly by the United Nations Development Programme, the World Bank, and the World Health Organisation in close contact with the pharmaceutical industry.⁸⁻¹³⁻¹⁴

Much information has now been collected on the pharmacology of mefloquine, a quinolinemethanol and the nearest alternative to chloroquine. This highly effective compound (Lariam) was discovered 10 years ago by the Walter Reed Army Institute of Research, synthesised and tested by a pharmaceutical company, and clinically assessed in Brazil,

Thailand, Zambia, and other countries. It is now being gradually introduced for treatment of malaria in combination with sulphadoxine and pyrimethamine (to prevent or delay the development of resistance to it). Sulphadoxine with pyrimethamine and quinine (alone or combined with tetracycline) remain of value for severe or chloroquine resistant infections with *P falciparum*. Nevertheless, chloroquine is still useful wherever resistance is absent or of low degree.

When it comes to the choice of the best and least toxic drug for chemoprophylaxis the views of experts are divided.¹⁵ Several new compounds are undergoing trials; these include halofantrine (a phenanthrene methanol related to mefloquine), certain pyridine methanols, artemisinin (a sesquiterpene lactone, the component of the Chinese Qinghaosu) and its derivatives, some triazines, pyronaridine, naphthoquinones, and derivatives of primaquine.¹² None of these compounds is yet ready for extensive clinical trials.

Methods for "targeting" primaquine by various carrier systems, such as liposomes or glycoproteins, are being explored, and the search for active antimalarials of plant origin has been intensified. Much hope has been expressed that our increasing knowledge of the biochemical receptors of plasmodia will help in the development of successful drugs.¹²

Vaccines

Despite some justified optimism, the rate of advance in the chemotherapy of malaria has been slow in comparison with the steady spread of drug resistance. Both the chemical synthesis of active candidate compounds and their field trials are fraught with difficulties, long on clinical evaluation, high on investment, and chancy on final expectations because of

FIG 1—World distribution of resistance of *P falciparum* to chloroquine in 1984 (WHO data). Each point represents confirmed focus of resistance.

the inherent possibility of plasmodial resistance to new compounds—unless they represent a completely novel departure in terms of biochemistry and pharmacology.

No wonder, then, that many scientists have turned to the promise of a malaria vaccine. The early history of vaccination against malaria has been told before.^{16 17} Recent international meetings have been devoted largely to the scientific and technical problems of vaccination against malaria and to clinical trials of prospective vaccines.¹⁸⁻²²

The plasmodium undergoes progressive transformations during its progress from the infective sporozoite injected by the bite of a mosquito, through the enormously amplified exoerythrocytic schizogony in the liver of the vertebrate host, followed by the cascade-like multiplication of the asexual stage in the blood, and then the appearance of sexually differentiated gametes, which proceed, after fertilisation, to the sequence zygote, ookinete, oocyst and to the formation of countless new sporozoites in the body of the insect vector (fig 2). Each of these stages has a well defined function, but,

ranging from 280 000 to 45 000 can now be obtained in a pure state through affinity chromatography using monoclonal antibodies, polyacrylamide gel electrophoresis, and other advanced biochemical methods. Furthermore, large scale plasmodial culture systems can now produce gram quantities of crude plasmodial antigens.

Further steps comprise cloning of genes that code for the protective antigens, analysis of their nucleotide composition, deduction of the amino acid sequence of the encoded molecule, and production of the relevant peptides by recombinant DNA methods or by solid phase synthesis.

Much of this preliminary work has already been achieved in several plasmodial species including *P falciparum*, and undoubtedly a candidate vaccine against human malaria will be developed before long. Indeed three types of vaccine against *P falciparum* are under investigation, and their eventual uses can be foreseen.

A merozoite or (more accurately) asexual erythrocytic vaccine, inducing immunity against blood forms of the

FIG 2—Cycle of development of malaria parasite. (Reproduced from *Essential Malariology*, second edition,¹¹ by permission of the publishers.)

though each has the same complement of genes, every stage expresses a different part of the plasmodial genome, and during this series of events the parasite generates an enormous number of antigens. Some of these stimulate the protective immune response of the host, while others are immunologically irrelevant or harmful. The production of a malaria vaccine will require the identification, characterisation, and use of those antigens which stimulate effectively the protective response of the host against a homologous parasite species. Although the principle of this procedure was known a long time ago, its practicability became obvious only in 1976 when Trager first succeeded in maintaining in vitro cultures of *P falciparum*,²³ while Milstein and Köhler developed the hybridoma technique of producing sensitive monoclonal antibodies.²⁴

Generally the path of development of malaria vaccine starts with identifying the antigens which induce the greatest specific immune response in animals (together with an absence of toxic effects). Next studies are carried out in primates susceptible to infection with human plasmodia. Very many isolated antigens of relative molecular weights

parasite, would act as a therapeutic compound and meet the problem of drug resistance; a sporozoite vaccine, preventing the infection, might replace chemoprophylactic compounds and be useful for protection of non-immune visitors to malarious areas and some vulnerable groups of indigenous populations; a gamete reactive vaccine used on a large scale might prevent the transmission of malaria in epidemic areas or where the danger of resurgence is high.

Sporozoite vaccine

Discovered in 1978 by R and V Nussenzweig and their group at the New York University, the main protective antigens of the sporozoite stage of the malaria parasite are concentrated on its surface and form a sheet-like precipitate (circumsporozoite reaction) when exposed to specific immune sera. The circumsporozoite proteins of several species of animal and human plasmodia react with a corresponding monoclonal antibody and have been designated by their plasmodial species and the antigen's relative molecular

weight. The circumsporozoite molecule plays a part in the penetration of sporozoites into the liver cells; corresponding monoclonal antibodies inhibit this entry.²⁵ It appears that the circumsporozoite protein is produced in relatively large amounts in the body of an infected mosquito; it is specific to the stage of development and to the plasmodial species.

The most promising development with regard to the sporozoite vaccine has been cloning the gene of the simian *P knowlesi* into *Escherichia coli*. This was done by extracting the messenger RNA encoding the circumsporozoite protein from infected mosquitoes, converting it (by the enzyme reverse transcriptase) into cDNA; the fragments of cDNA were inserted into bacterial plasmids, which were then introduced into *E coli*. The bacterial clones excreted peptides binding to the monoclonal antibodies against the circumsporozoite protein of *P knowlesi*.²⁶ Moreover, the peptide corresponding to the relevant sequence of nucleotides of *P knowlesi* was synthesised, coupled to a protein carrier with an adjuvant, and injected into animals; these responded by forming anti-peptide antibodies and neutralised infective sporozoites.

Sequencing analysis of the circumsporozoite protein showed that the immunodominant antigenic determinant (epitope) contains a sequence of 12 amino acids tandemly repeated 12 times. When synthesised this molecule reacted with the monoclonal antibody to the original circumsporozoite protein. The gene has also been inserted into vaccinia virus; cells infected with the recombinant virus produced the sporozoite antigen, which was immunogenic.²⁶

Another advance has been cloning and sequencing the gene coding for *P falciparum* circumsporozoite protein.²⁷ This was achieved recently by two groups of workers in New York and Washington using two different methods. The sequencing analysis of the immunodominant epitope thus obtained showed that it contained repeated sequences of short chains of amino acids—the subunits of protein. The peptides were synthesised and shown to share common antigens with the original circumsporozoite protein.²⁰

Asexual erythrocytic vaccine

The main advantage of a blood stage vaccine would be its therapeutic value; even if it did not induce complete immunity it might be useful in severe infections. Cohen's early work showed that non-living plasmodia when given in a proper antigenic form combined with adjuvant induced a better immune response than living parasites, which while eliciting a degree of defence immunity also activate processes that favour their survival.¹⁶

Possibilities for development of blood stage vaccines have greatly increased with the recent remarkable advances of immunochemistry and the availability of a wide range of monoclonal antibodies, together with in vitro cultures of *P falciparum*. The two main targets for an asexual erythrocytic vaccine are, firstly, the free merozoite circulating in the blood and, secondly, the infected erythrocyte. Analysis at these sites has shown that there are very many antigenic proteins. Some may be recognised by antibodies which block plasmodial growth, while others agglutinate the merozoites. Systematic comparison of various antigens of *P falciparum* is now in progress.¹⁸

Immunisation of animals with purified antigens from the surface membrane of the blood stage parasite confers substantial protection against subsequent challenge. Mono-

clonal antibodies have been produced against *P knowlesi* and *P falciparum*, which react with high molecular weight antigens of schizonts and merozoites. A new class of malarial antigens has been identified which binds specifically to glycophorin, the surface component of erythrocytes; possibly these antigens are concerned in the penetration of merozoites into the blood cells since specific antibodies affect the parasite growth.

Another large group of soluble, heat stable antigens of *P falciparum* are released in the serum or into the culture medium and have recently been characterised with regard to their amino acid sequence.²⁸ Several high molecular weight purified polypeptides produced during schizogony can protect monkeys from fulminant parasitaemia and are also potentially useful immunogens. The recent cloning of *P falciparum* blood stage antigens into *E coli* through bacteriophage into which the plasmodial cDNA has been inserted was a notable achievement.²⁹ The expressed peptides were reactive with antibody from human immune sera collected in Papua New Guinea.

Yet with all the progress in developing an asexual blood stage vaccine against human plasmodia their antigenic diversity presents many difficulties. Future studies will have to define the antigen most likely to give protection and identify its relation to specific pathological changes produced in man.

Gamete related vaccine and exoerythrocytic stage immunisation

The sexual stages of malaria parasites are concerned in the transmission of the infection by the mosquito vector. Antibodies induced in the vertebrate host against gametocytes may act against the development of further stages in the mosquito. This has been shown in several plasmodia, including *P falciparum*; as a result the transmission of the infection is blocked when the antibody is ingested by the mosquito in the course of its blood meal. Antigens reactive with specific monoclonal antibodies have been identified and are present in male and female gametes. They are also present in the gametocytes but expressed fully on the surface of later sporogonic stages. Possibly DNA recombinant technology may lead to the development of a transmission blocking immunogen which would eventually form a component of a polyvalent malaria vaccine. The recently developed methods of cultivation in vitro of exoerythrocytic liver forms of mammalian plasmodia have opened the way for studies of their antigenic composition and their part (if any) in inducing an immune response.

All three prospective vaccines, targeted at different stages of the cycle of development of malaria parasites, have their own advantages and disadvantages. The work on the sporozoite vaccine is most advanced and seems certain to be produced and tested first, but its inherent defect is its "all or none" effect: if it fails to prevent infection the disease may proceed without much change in its course. An effective asexual blood stage vaccine may produce a rapid cure or at least result in a less severe illness. The potential value of the gamete related vaccine is more conjectural, but it would be useful for curbing epidemics or seasonal outbreaks of malaria. Perhaps a polyvalent vaccine might fulfil all three needs.

As the immunogenic effects of any of the three prospective vaccines will be limited they will require a carrier molecule and an adjuvant. Vaccinia virus is a large DNA virus

previously used for smallpox vaccination, and techniques have already been developed for inserting genes of other infectious agents (hepatitis B, herpes simplex, influenza, rabies, and so on) into the virus genome. Vaccinia virus recombinants have been shown to express the circumsporozoite antigens of *P knowlesi* and *P falciparum* and to induce antibody responses in animals, enhancing both the humoral and the cell mediated immunity.³⁰ Any vaccine would also require the addition of an appropriate adjuvant to potentiate the immune response, reduce the amount of antigen needed, and stimulate the synthesis of various immunoregulatory lymphokines. Adjuvants such as saponin, muramyl peptides, and others, used in testing the candidate vaccine in animals, cannot be employed in man. The search for a suitable adjuvant is now intensive in many research institutes.^{31 32}

The expectations from the future malaria vaccine are high—perhaps too high. We hope that it will be specific against all strains of *P falciparum* and yet partly active against other species of human plasmodia; the duration of the potent immune response should be at least a year and preferably much longer; it should protect not only newcomers to the tropics but also people partly immune to malaria; it should cause no, or the least possible, side effects, whether general or local, when given to people of any age and of either sex; it should be stable at fairly high environmental temperatures and require no cold chain storage; and it should be easily

administered, reasonably easily made in bulk, and affordable by tropical countries—a tall order.

Addendum

Two studies published this year indicate the speed with which research on malaria vaccines is proceeding. A paper published on 17 May in *Science* reported that the circumsporozoite protein, a surface antigen of the simian *P knowlesi*, was cloned and expressed in the yeast *Saccharomyces cerevisiae* by using an expression carrier containing a regulatory region of the yeast gene for the alcohol dehydrogenase.³³ The same month *Science* published a paper reporting that the recombinant product of an expression of circumsporozoite protein in *P falciparum*³⁴ in the bacterium *E coli*, when injected into animals, stimulated the formation of specific antibodies, which reacted with the circumsporozoite protein of live sporozoites of this plasmodium and blocked their in vitro invasion of human hepatoma cells, thus confirming the candidacy of circumsporozoite protein as a material for a human malaria vaccine.

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