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LEVEL II

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PROCEEDINGS OF THE ~~FIRST~~ MEETING OF AUSTRALIAN RESEARCH WORKERS ON MALARIA (1st)

Held at

INGLEBURN, 22-24 ~~FEBUARY~~ February 1980.

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DIRECTORATE GENERAL OF ARMY HEALTH SERVICES

CANBERRA, 1980

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I. INTRODUCTION

A Meeting of Australian Research Workers on Malaria took place at the Military Camp at Ingleburn, NSW, from 22 to 24 February 1980. It was convened by the Director-General Army Health Services and sponsored by the Australian Army First Malaria Research Unit.

The purpose of the meeting was:

1. To acquaint each participant with the details of research work on malaria, or closely related subjects of possible relevance to malaria, being carried out at present by other research workers, of the facilities available to each of them (models, strains, equipment, skills, experience), and of the short and long-term plans of research, in order to define possible areas in which collaborative efforts might appear fruitful or advantageous, and to avoid duplication of efforts unwarranted.

2. To review the main facets of malariology requiring additional research efforts, possibly of a multi-disciplinary nature, for overcoming the present difficulties that are being faced in the prohylaxis, control and treatment of malaria, and for elucidating specific problems in the life-cycle of the malarial parasite, its enzyme patterns, the pathology of the disease, the mode of action of drugs and the epidemiology of the disease.

3. To draft proposals for a collaborative research programme and for repeated consultations.

Participants were invited from various Universities of Australia and from Australian and Papua New Guinea Institutions which were known to carry out basic research on different aspects of malaria (epidemiology and control, immunology, biochemistry and enzymology, entomology, pathology, genetics, etc) in the laboratory or in the field in the form of applied research. A few research workers on allied subjects with some affinity to malaria (eg babesiosis) were also invited, as well as a representative from the World Health Organisation, in view of the overall co-ordination role that befits that organisation, and representatives of the Australian Society of Parasitology.

The response to the invitation was most gratifying, 38 participants attending, and two more sending in their written contribution as they were unable to attend in person.

In order to foster the mutual acquaintance, each participant (or group of participants working together in the same Institution) was requested to present at the meeting a short summary of his/her present research endeavour concerning malaria, of the facilities available (strains, models, equipment, specific experience), the short and long-term plan of research, as well as an indication of possible areas of their work in which collaborative effort may prove advantageous as regards malaria research.

The research endeavours related to malaria include: epidemiology and control, diagnosis, cellular biology, bio-chemistry and enzymology, clinical pathology, immunology, structure and effect

of drugs, drug-resistance, vector speciation and distribution, biological control. These summaries were distributed to participants and were spoken to briefly at the meeting. They constitute Annex B to the present report.

The meeting then considered in four separate working groups the following aspects of malaria research open to a multidisciplinary approach, and of interest to both laboratory research workers and field research workers, namely:

a. Diagnosis of malaria.

b. Epidemiology and control of malaria.

c. Pathology and clinical aspects of malaria.

d. Prophylaxis and treatment of malaria.

The discussions of and the proposals arising from the working groups were discussed in a plenary session of the meeting, and are summarised in Part II of this report.

Finally the plenary session made a unanimous recommendation to the Australian Army concerning future meetings of this type. (Part III of this report).

The list of participants is given in Annex C, with their individual position and addresses; the list of Institutions represented at the meeting is given in Annex A.

Office holders at the meeting were the following:

Plenary Sessions

Convener : MAJGEN W.J. Watson (Canberra)

General Chairman : DR I.A. Musgrave (Brisbane)

General Rapporteur : LTCOL A.D. Parkinson, (Ingleburn)

Secretary : DR G. Gramiccia (Ingleburn)

Group Sessions

Group 1 : Diagnosis of Malaria

Chairman : DR G. Brown (Melbourne)

Rapporteur : DR R.A. Cooke (Brisbane)

Group 2 : Epidemiology and Control

Chairman : DR R.B. Hawes (Brisbane)

Rapporteur : DR D.L. Houghton (Sydney)

Group 3 : Pathology and Clinical Aspects

Chairman : DR P. Cavanagh (Melbourne)

Rapporteur : DR J. Stace (PNG)

/Group 4

Group 4 : Prophylaxis and Treatment

Chairman : PROF R.H. Black (Sydney)

Rapporteur : DR C. Bryant (Canberra)

The meeting was opened by DGAHS, Major General W.J. Watson, who delivered the following address:

I.1 Opening Address by MAJGEN W.J. Watson, MBE, QHP
Director-General of Army Health Services

"It is extremely gratifying to see such a wide representation from various parts of Australia, Papua New Guinea, World Health Organisation in Manila, and I extend to you a most genuine welcome on behalf of the Chief of the General Staff and the Australian Army at large, as well as my warmest personal regards.

Just one month ago a team of Consultants from the World Health Organisation was in Australia to certify that malaria had been eradicated from this country. While this may be a fact, the pressure of malaria around this continent is, nevertheless, on the increase, especially from drug-resistant strains of *P. falciparum*. Last month a young man died in Australia of malaria contracted abroad. In 1979 there were 443 cases of imported malaria, and *P. falciparum* was found in 130 of them. The same mosquito, *An. farauti* No. 1, that carries malaria in Papua New Guinea, has recently been identified in the Northern Territory. Australia is under a constant threat of re-invasion by malaria. In addition, travellers abroad are often at risk, and of course the Armed Forces are always potentially at great risk. This is our raison d'etre for the re-establishment and maintenance of the Army Malaria Research Unit which first came into being, albeit under a different name, in Cairns in 1943.

Many research workers in Australia are engaged in very high level investigations on various scientific aspects of malaria : immunology, pathology, entomology and control, toxicology, prophylaxis, treatment, biochemistry and genetic aspects. A few people are coordinating the hard field work of surveillance of malaria over the National Territory.

This meeting is intended to offer the occasion for an encounter between these two categories of malaria workers. The first may tell us what they are doing, and what they expect from their difficult, inquisitive work, that may prove of consequence, now or later, for the control of malaria. The others perhaps, might say what they would need most, now, for their daily work, and how research could help them. In addition, participants will have the opportunity to get to know each other better, and to become acquainted with each other's work and facilities.

We all hope that some new ideas, some priorities, some useful forms of collaboration may stem from the group discussions. This meeting hopefully will help clarify present and future trends of research and their relation to present needs. Researchers working on other subjects that could be related to some aspects of the malarial infection, may stimulate our awareness of different and important approaches to the

problem.

This meeting is not intended to be a forum for the discussion of the merits or otherwise of the purely technical aspects of current research projects. Quite apart from any other considerations, there simply isn't time for that.

Personally, I believe that if after the meeting, each one of you is stimulated to think over his own programme in the light of what has been said during the sessions, the meeting will have achieved its main purpose.

I regret very much that circumstances have prevented the Army from making a greater contribution to this gathering and I pay tribute to your interest and dedication for I realise that you are here at considerable personal inconvenience and expense.

I thank you most sincerely for your generous contributions to this gathering, and wish you fruitful discussions.

I now invite Dr Musgrave to take the chair."

II. SUMMARY OF THE DISCUSSIONS ON SELECTED SUBJECTS OF RESEARCH ON MALARIA

Two general considerations governed the discussions. The first was that the short duration of the meeting did not leave sufficient time for a thorough discussion of the subjects in all their aspects : only a few points of interest could be considered for each subject and these were usually discussed with reference to the type of work currently carried out by the participants.

The second was that in discussing these subjects, three main criteria should be kept in mind, namely:

a. the recognised priority demands for research in each of the selected subjects,

b. the lines of research that were most likely to produce an advance in the knowledge of the subject and that could lead to the practical application of its eventual result,

c. the identification of areas of research in which collaboration would be useful, given the experience and the facilities at present available in various institutions in Australia.

The following subjects were discussed in separate working groups and the summary of the groups' debates and conclusions were then presented to the plenary session of the meeting for endorsement. The following represents therefore a consensus of opinions.

II. 1. DIAGNOSIS OF MALARIA

An accurate diagnosis of malaria is essential for the clinical treatment of patients, for epidemiological studies as well as for

most research activities on malaria.

II. 1.1 Microscopical diagnosis

At present, the only universally acceptable criterion for the confirmation of diagnosis of malaria is the microscopical detection of the parasite either directly in a stained blood film or at the limit as a result of sub-inoculation. In the absence of demonstrable parasites even after repeated blood film examinations no confirmation of on-going malarial infection can be given.

The accuracy of the microscopical diagnosis depends on the quality of the laboratory services available and on the experience of the microscopist; it may vary widely. Data from the National Reference Centre at the School of Public Health and Tropical Medicine in Sydney suggest that the quality of diagnosis of malaria in laboratories in Australia is variable. This does not take into account the number of positive cases that may be missed initially but considers only blood films claimed as positive, and the species diagnosis.

There is a need on the one hand for improvement in the accuracy of the diagnosis of malaria, including species diagnosis, by laboratories and on the other hand for an improvement in the methodology to ensure a simple, rapid and accurate test which can be used in the field or at the patient's bedside as well as in the laboratory.

II. 1.2 Other Methods of Laboratory diagnosis

II. 1.2.1. Presently used Tests

Immunofluorescence Test (IFT)

This test can be used to measure previous exposure to malaria. It is currently used in epidemiological surveys as an indicator of previous infections in the population, but is unsuitable for the diagnosis of the acute infection or for measuring protective immunity. It takes months before a new infection produces a positive IFT. The test can differentiate, based on titres, antibodies to the three major species of human malaria parasites, but not different strains.

Enzyme-linked Immunosorbent Assay (ELISA)

This test, as with the previous one, assesses antibodies and the same comments apply as to the above. Although the interval between the acquisition of the infection and the positive result of the test is shorter than with IFT, it remains too long (several weeks) to be useful for diagnosis of an acute infection. This test also presents some problems of non-specific binding of antibody giving a high background reading. More purified antigen preparations may provide more specific and reliable readings.

II. 1.2.2. Tests that could be developed

Hybridoma Reagents

Monoclonal antibody to parasite antigens could be the basis of

/highly specific

highly specific tests applicable to the diagnosis of individual patients with malaria, and for epidemiological studies. Such reagents could permit species and possibly strain diagnosis.

Biochemical Tests

Identification of enzymes present in the metabolic pathways of the parasite and not present in the uninfected red cells could be used as a diagnostic test for parasitaemia.

II. 1.2.3. Diagnosis of chloroquine resistance in *P. falciparum*

The diagnosis of chloroquine resistance is made following the recrudescence of *P. falciparum* or a partial or no response following standard chloroquine therapy, or by in vitro sensitivity testing of infected blood. In vitro testing is being performed in 2 laboratories at present in Australia and a third is tooling up for the test. The macro-method is the test commonly used and a micro-method is being developed. Certain technical problems remain to be resolved and laboratories are collaborating to standardise procedures so that results will be comparable. Both in vivo and in vitro tests can be used for epidemiological surveys in the field and in the management of individual patients. The in vitro test is invalidated if antimalarial drugs have been given to the patient whose parasites are being tested and is limited by the degree and maturity of the parasitaemia. Every effort should be made to draw blood for testing prior to treatment being given. Sufficient blood should be taken and defibrinated so that a 10 ml sample can be shipped sterile, on ice to 1 MRU. Such a course may save on therapy as it will indicate the likelihood of recrudescence.

The World Health Organisation will provide test kits for in vitro sensitivity testing on request. It is hoped that the recent micro-technique can be used widely in field surveys.

II. 1.4 Priorities for action:

a. The reliability of microscopical diagnosis of malaria needs improvement. It is dependent upon the training of individual microscopists. It was recognised that it takes several weeks of repetitive blood film examinations, for a new recruit to become proficient in positive/negative and species diagnosis and several months to reach a high degree of accuracy. It was agreed that a training scheme for laboratory workers (microscopists) should be set up in Australia on a regional (State) basis. One centre in each State should undertake to arrange periodical training and in-service re-training of staff from public and private sectors in the technique of preparation of slides and of microscopical diagnosis of malaria. An expert from the School of Public Health and Tropical Medicine could serve, as warranted, as a resource person to such courses. The courses could then be taken to other centres within each State. The College of Pathologists could also be approached to ensure that all positive slides continue to be sent to SPH & TM for diagnostic confirmation or for surveillance purposes.

b. Immunological tests offer prospects for becoming specific, sensitive diagnostic tools in malaria. The development of specific anti-parasitic antibodies by hybridoma techniques, already set up in some Australian Institutions, offers an expectation of important advances in the diagnosis of acute infections as well as in epidemiological surveys and deserve encouragement.

c. The development of biochemical tests is possible; however, the expectation of their practical application in the immediate future appears small.

d. The monitoring of the spread of chloroquine resistance should receive high priority, and the comparability of the result of tests carried out by different institutions using macro, micro and in vivo methods should be assessed further. The in vitro assessment of chloroquine resistance should receive consideration in the management of every case of falciparum malaria in Australia. A brief history of the patient's present malaria infection and the date, type and dosage of the last anti-malarial drug taken should accompany each sample of blood for testing, preferably together with a small sample of urine collected at the same time as the blood sample.

II. 1.5 Collaboration

Considerable advantage could result from collaboration between laboratories and Institutions in Australia, and all groups have indicated their willingness to collaborate. The following list indicates those that are using at present methods and techniques of consequence for the diagnosis of malaria:

a. Microscopical diagnosis of malaria, training and in-service re-training in this technique (Regional Laboratories):

- School of Public Health and Tropical Medicine, Sydney. (This is the central reference centre to which all positive slides diagnosed in Australia should continue to be sent for confirmation and registration).

- Army Malaria Research Unit, Ingleburn.

- Fairfield Infectious Diseases Hospital, Melbourne.

b. Immunofluorescence (IFT)

- School of Public Health and Tropical Medicine, Sydney.

- Faculty of Medicine, University of Newcastle, NSW.

c. Enzyme-linked immunosorbent assay (ELISA)

- Papua New Guinea Institute of Medical Research, Madang.

- Queensland Institute of Medical Research, Brisbane.

/d. Long

d. Long-term in vitro cultures of *P. falciparum*

- Walter and Eliza Hall Institute, Melbourne.
- Queensland Institute of Medical Research, Brisbane.
- Faculty of Medicine, University of Newcastle.

e. In vitro testing of drug-resistance

- 1 Army Malaria Research Unit, Ingleburn.
- Queensland Institute of Medical Research, Brisbane.
- Fairfield Infectious Diseases Hospital, Melbourne.

f. Hybridoma technique

- Walter and Eliza Hall Institute, Melbourne.
- Queensland Institute of Medical Research, Brisbane.

The above list has been drawn from the personal knowledge of participants. There may be other laboratories and institutions also in other States in Australia that may be willing to collaborate.

II. 2. EPIDEMIOLOGY AND CONTROL OF MALARIA

The topics selected for discussion were:

a. the proposal for a malaria research and training facility in the Torres Strait Area,

b. the biological control of vector mosquitoes with particular reference to the fungus *Culicinomyces,*

c. the chemoprophylaxis programme, with reference to the problem of drug resistance.

II. 2.1 The Torres Strait Malaria Research and Training Facility

The Torres Strait Islands are maintained free of malaria by a modified vigilance system supported by a single annual round of indoor spraying with DDT and the screening of arrivals from PNG. There is some doubt as to whether the measures are economically appropriate; baseline entomological studies are required to assess the effectiveness of DDT spraying. In addition, considerable concern was expressed on the paucity of available data on sibling species of anopheline vectors, particularly *A. farauti,* their distribution and behaviour in Australia. However, it was pointed out that freedom from malaria had been attained in Australia with little entomological background research and perhaps of more importance to the malaria status of Torres Strait is the study and monitoring of population movement, or more likely, a combination of both studies. Entomological parameters are however essential in any malaria epidemiological study.

The Torres Strait Research post should be set up as a practical component of the management of malaria vigilance, as well as a

research facility. This would provide a back-up service for the Queensland Health Department presently carrying out vigilance operations in the area. The School of Public Health and Tropical Medicine wishes to train malariologists, and needs therefore to set up a scheme to train post-graduate students from Australia and elsewhere. The project would therefore involve also a teaching aspect, with training in maintenance phase activities. This would have relevance also for other countries, especially in the South-West Pacific region.

II 2.2 Biological control of vector mosquitoes

A number of biological agents and procedures have been tried in various areas of the world for the control of malaria vectors. Amongst the fungi, *Culicinomyces* sp. studied at 1 MRU has a larvicidal effect on a narrow range of insect species and might be applied in a manner similar to that used for chemical larvicides, without the problem of ecological pollution related to chemical insecticides. However 30°C has been found to be the upper limit to growth of the fungus. This might constitute a limit to its effective application in some tropical areas. There is also the problem of its fairly rapid sinking from the surface, where anopheline larvae feed.

Extensive research and field trials on *Culicinomyces* sp. are being pursued.

II. 2.3 Chemoprophylaxis programme

A description of the chemoprophylaxis research programme in Madang province, PNG was presented. Weekly amodiaquine prophylaxis is provided to children 0-5 yrs; as children grow into the next age-group, they are given amodiaquine for treatment only when they have fever. As resistance of *P. falciparum* to 4-aminoquinolines is present and on the increase in PNG, concern was expressed that drug pressure would exert a selective effect upon strains of *P. falciparum* resistant to chloroquine. A number of alternatives were proposed to that of discontinuing the research. These were:

a. Simultaneous administration of primaquine to slow down the development of resistance.

b. Short term mass administration of Maloprim to the total population.

c. Other measures combined with the amodiaquine programme:

(1) environmental sanitation.

(2) DDT spraying conducted by the community itself.

These alternatives were discussed by the participants.

Primaquine distribution might neither be acceptable nor advisable (in view of G6PD deficiency). However, in view of insufficient data on the distribution and type of G6PD deficiency, and of the usually rapid recovery from haemolysis due to a single

dose of primaquine, the use of this drug should not be excluded altogether.

Whilst short-term Maloprim might diminish or remove resistance in one locality, the strain would be re-introduced from neighbouring areas. In addition, the possibility of Maloprim resistance would also arise.

Environmental sanitation would have little application in rural areas where malaria presents the greatest problem, because of the nature of the breeding sites of the vectors.

There would, however, be a case for combined mass drug administration with spraying operations conducted by the community. Whilst an objection was made that the combination would complicate research in so far as answers were sought for the role of chemoprophylaxis alone, it was felt that a combination of MDA plus spraying would be a worthwhile inclusion within the present research project.

II. 2.4 Conclusions

The meeting strongly supported the proposal which has been made by the School of Public Health and Tropical Medicine for the establishment of a Malaria research and training facility in the Torres Strait area. The setting up of a post to this effect is a priority not only for responding to the strongly felt need for epidemiological research and training in malaria, but also for assisting in the maintenance of Australia's malaria-free status.

Methods of biological control of vectors may provide acceptable techniques without the ecological problems associated with chemical insecticides. Support of research on biological control methods should however go hand-in-hand with that of chemical insecticides that play and will continue to play a very important role in vector control.

Larvicides in general have been found useful for malaria control in urban areas. However, in some countries larval control could be feasible also in rural areas, and more emphasis should be placed upon research on the practicability of the use of larvicides for malaria control in those areas.

Priority should be given to research for the genetic selection of the fungus *Culicinomyces* sp. for growth at temperatures higher than 30°C, as well as for the development of appropriate formulations that would prolong the period of floating of the spores on water surfaces and consequently the duration of the period of the effective attack of the fungus against anopheles larvae.

II. 3. PATHOLOGY AND CLINICAL ASPECTS OF MALARIA

Three main subjects were discussed:

a. anaemia in malaria,

b. cerebral malaria, and

c. toxins in malaria.

II. 3.1. Anaemia in Malaria

Malaria may cause acute or chronic anaemia. The degree of anaemia may be more severe than is suggested by the number of parasitized RBC's. The anaemia may be caused by three factors:

a. Haemolysis of parasitized and non-parasitized RBC's.

b. Sequestration of RBC's.

c. Depression of bone marrow function.

In chronic malaria, the iron from destroyed RBC's is apparently not available for haemopoiesis and may be eliminated with the urine. In mice, uninfected RBC's seem to be removed from circulation as well as the infected cells.

In considering the question of autoimmune antibodies, it appears that although cold agglutinins are detectable in patients with chronic malaria and their amount increases with the age of infection, they probably do not harm the RBC's.

Clinical experience was presented of a small group of patients with anaemia secondary to malaria. The anaemia was severe, and did not improve until the patients received steroids. It was suspected that this was due to an autoimmune haemolytic anaemia but no antibodies were detected on the RBC.

There is the possibility that the non-parasitized RBC's are passively coated with soluble parasite antigen secreted by parasites in infected RBC's.

II. 3.2 Cerebral Malaria

Cerebral malaria is usually, but not always, associated with fever and high *P. falciparum* parasitaemia. The CSF is normal. It was suggested that surface protrusions in parasitized RBC could enable the cells to adhere to the capillary endothelium thus causing cerebral malaria. This has not, however, been borne out by recent electron micrograph studies of infected placentae and may need further clarification.

An animal model for cerebral malaria studies in the golden hamster has been described in the literature and this could possibly give some answers to some of the questions posed by this syndrome.

II. 3.3 Toxins in Malaria

The mouse malaria model (*P. vinckei petteri*) was described. It exhibits hypoglycaemia, poor blood clotting, focal necrosis in the liver and death of cells in lymphoid organs. A "crisis" may be stimulated in mice with about 20% parasitaemia following the administration of small doses of endotoxin lipopolysaccharide (LPS). A comparable reaction may occur in man, and studies along this line may provide some answers to problems associated with human malaria.

II. 3.4 Proposed research

It was agreed that the possibility of parasite antigens or

antigen/antibody complexes on the surface of uninfected RBC's in malaria patients should be further investigated in relation to malarial anaemia.

Parasitised blood could be collected from patients with cerebral malaria in PNG and sent to interested laboratories in Australia for cultivation and further study after surface labelling of parasitized red blood cells.

Other selected topics for further study which could not be discussed in the time available but which are considered of some importance include:

a. The effects of chemotherapy on the parasite/host complex.

b. Antitoxins and endotoxin antibodies, a subject studied by Jerusalem some years ago, require follow up.

c. The virulence in man of chloroquine resistant strains of *P. falciparum*.

d. The extent, if any, of cross-immunity in malaria due to strains of *P. falciparum* respectively susceptible and resistant to chloroquine.

II. 4. MALARIA PROPHYLAXIS AND TREATMENT

4.1 With the emergence of resistant strains of malaria in various parts of the world, including the SW Pacific area, there is need for alternative drugs for prophylaxis, treatment and methods of administration of drugs.

II. 4.2 Subjects discussed and proposals made

a. Apparent failure of prophylaxis

It was agreed that, whenever the suspicion arises of the possibility of resistance of a malaria infection to a chemotherapeutic agent, either administered prophylactically or therapeutically, detailed histories of the affected person should be collected and scrutinized prior to deciding upon change of prophylaxis or detailed drug studies. This scrutiny should include the microscopical confirmation of the parasite species involved, the method of administration of the drug (supervised, unsupervised), the type and amount of drug administered, and the presence of any factor capable of altering the absorption of the drug (eg gastro-intestinal troubles, vomiting, etc).

b. Possible Inadequacy of Maloprim

There was considerable discussion on the alleged failure of maloprim to prevent vivax malaria. Conflicting views were voiced about the value of the hearsay evidence and alternative explanations were offered - such as failure of patients to observe the drug regime, or misdiagnosis of the malaria type. Because Maloprim is being widely used in PNG where chloroquine-resistant *P. falciparum* is

prevalent, it was felt that such anecdotal evidence of failure should be confirmed or refuted on reliable evidence. A simple urine test for Maloprim does not exist; although there is a blood test for its components, it is too complex to be used for screening in the field. It was therefore agreed that there was a need for controlled experimental work on Maloprim and its capacity for prophylaxis in chloroquine-resistance areas and in areas of vivax malaria. If break throughs occur, what species or strain of parasite is involved? The Army may be able to carry out such a trial, and studies on the long term toxicity of the drug could also be undertaken concurrently or separately.

c. Dapsone

Dapsone has a short biological half-life but is still effective in combination with pyrimethamine (Maloprim). Little is known about its mode of action and that of Maloprim or how far apart the doses may be administered and still retain their full antimalarial effect. This should be investigated. Further, depot doses of a sulphone (DADDS) are used in treating leprosy and have also shown to have an effect on *P. falciparum*. There may be a possibility of using this form of sulphone administration in association with oral pyrimethamine, given regularly between injections.

d. Chloroquine plus Maloprim

A comparison of the efficacy of Maloprim and of Maloprim and chloroquine could be carried out in a vivax area, as well as in areas where *P. falciparum* is not resistant to chloroquine.

e. Fansidar

This drug has been used for up to 2 years for prophylaxis. No breakthroughs were observed in one study lasting one year in Laos with a prevalence of chloroquine resistant falciparum. No side effects were observed, except for a mild reversible leucopoenia in 10% of 200 subjects. There is a need for large trials to determine if more severe complications occur.

f. Sulphonamides and Bacterial Resistance

There is concern lest prolonged use of sulphonamides for malaria prophylaxis may induce resistance in bacteria, as a side effect. Further evidence is required.

g. New Antimalarials

Mefloquine, a 4-quinolinemethanol has been widely used in clinical trials in Thailand, and should be available for marketing in Australia in 2 years time. Two new drugs are under development in China, one of which "qinghaosu"

/is not

is not structurally related to those in current use, and may provide a new drug nucleus from which further anti-malarials might be developed.

h. Primaquine

It is not clear how the standard gametocytocidal dose of 45 mg was derived and this should be investigated both from literature and from field trials, as a smaller dose might be adequate. A study on this may be carried out by 1 MRU in collaboration with the Department of Health of the Solomon Islands. Concern about the effect of Primaquine on carriers of G6PD deficiency demands that careful consideration is given to the possible dangers before such a study is undertaken in areas where the trait is present.

i. Pyrimethamine and Proguanil

Tests of blood levels for those drugs are difficult to perform. Easier tests are required.

j. Maloprim, Fansidar and Pregnancy

Studies are required on the effects of Maloprim and Fansidar on the unborn child. They should not be used in pregnant women because the effects on the foetus and the new-born are not known. Sulfonamides may produce kernicterus in the new-born, and dapsone in high doses has been reported to have teratogenic effects in rats. Almost the only prophylaxis available to pregnant women in PNG is chloroquine. Retrospective studies should be carried out on women who have had babies whilst chosing to take Fansidar or Maloprim prophylaxis during pregnancy.

k. Quinine and Fansidar

This is the combination of choice for the treatment of chloroquine resistant falciparum malaria; but for how long after microscopic clearance of asexual parasitaemia should quinine be continued? This is particularly important in relation to past observations that in areas where *P. falciparum* is at present resistant to chloroquine, the parasite appeared to withstand doses of quinine that provided a satisfactory cure rate in other areas. The timing of the administration of Fansidar after quinine in this treatment was also discussed. There is no indication for injections of Fansidar in the treatment of malaria.

l. An Information Booklet

In order to keep individual Medical Practitioners up-to-date on the changing aspects of malaria treatment and prophylaxis, it was suggested that a booklet be devised outlining demographical and ecological aspects, as well as the distribution of chloroquine resistance. It was however pointed out that a number of alternative methods

of disseminating this information were at present being developed, in view also of the fact that one of the target groups (travellers) often do not seek advice from their doctor prior to travelling.

m. Diagnosis

When falciparum malaria is suspected, the greatest effort should be to collect blood before treatment and send it to 1 MRU for in vitro sensitivity studies. Such a course may save on repeated therapy as it will indicate the likelihood of recrudescence. Blood should be defibrinated, sterile and shipped on ice. (See also II 1.4.d.)

II. 4.3 Conclusions

There is a need for controlled experimental work on Maloprim and other malaria prophylactics where unconfirmed evidence suggests parasite breakthroughs. It may be possible for the Australian Army to conduct such trials in troops stationed in malarious areas or in human volunteers as was the case during the 2nd World War in Cairns. A comparative trial of Maloprim and of Maloprim plus chloroquine should be carried out in a predominantly vivax malaria area to assess whether Maloprim is any less effective against vivax malaria than the combination.

Because of the lack of satisfactory data on the long term effects of certain drugs on humans, the effects on the foetus in pregnant women and the effects on other pathogenic microbes there is a need for long term prospective and retrospective studies to be undertaken.

III. VENUE OF FUTURE MEETINGS OF AUSTRALIAN RESEARCH WORKERS ON MALARIA

At the end of the meeting, participants indicated the benefits they had derived from this first opportunity of becoming personally acquainted with the work of the various groups interested in malaria research in Australia and of the inter-disciplinary venues for collaboration that stemmed out of the discussions.

They expressed a strong wish that a meeting of this type be repeated at intervals of 2-3 years in order to learn and discuss the progress made in the different fields and to explore new openings for fruitful collaboration.

The question arose of the venue of future meetings.

The possibility that the Australian Society for Parasitology become the convener of such meetings was presented, but it was pointed out that the rotating venue of the ASP in a different State every year may constitute an impediment as the largest majority of research workers on malaria reside in Southern Queensland, NSW and Victoria.

A unanimous request was therefore addressed by the participants to the Director General of the Army Health Services to ensure that the Army continue to be the convener of the meetings on malaria research through the facilities available at 1 MRU, and that the responsibility for calling future meetings as required and providing the necessary secretariat be vested in 1 MRU.

The DGAHS indicated that there were no objections on his part to this request.

1 MRU will therefore remain available to all suggestions by Australian research workers on malaria as to proposals for future meetings and subjects warranting discussion or collaborative studies.

LIST OF INSTITUTIONS REPRESENTED AT THE MEETING

Geographical Area		Institution
1.	Brisbane, QLD	- Queensland Institute of Medical Research. - Department of Health - Aboriginal Health Programme.

2.	Canberra, ACT	- Australian National University.
		- John Curtin School of Medical Research
		  - Microbiology Department
		  - Department of Human Biology.
		- Research School of Biological Sciences
		  - Virus Ecology Research Group.
		- Department of Zoology.

3.	Darwin, NT	- Department of Health, Environmental Health Division - Entomology Section.
4.	Melbourne, VIC	- The Walter and Eliza Hall Institute of Medical Research - Laboratory of Immunoparasitology. - Fairfield Hospital (Infectious Diseases) - The Victorian College of Pharmacy.

5.	Newcastle, NSW	- The University of Newcastle, Faculty of Medicine
		  - Division of Clinical Investigations.
		- The Royal Newcastle Hospital
		  - Hunter Immunology Unit.

6.	Papua New Guinea	- Papua New Guinea Institute of Medical Research. - Madang General Hospital - Laiagam Rural Health Centre.

7.	Sydney, NSW	- The University of Sydney
		  - School of Public Health and Tropical Medicine (now Commonwealth Institute of Health).
		- The University of New South Wales
		  - School of Biochemistry.
		- The Australian Society for Parasitology.
		- Commonwealth Scientific and Industrial Research Organisation (CSIRO)
		  - Division of Animal Health.
		- Royal Australian Army Medical Corps
		  - First Malaria Research Unit, Ingleburn.
		- Wellcome Australasia.
		- Repatriation General Hospital, Concord.

8.	Western Pacific	- World Health Organisation - Regional Office for the Western Pacific, Manila, Philippines.

PRESENT STATUS OF RESEARCH ON MALARIA AND ALLIED SUBJECTS IN AUSTRALIA AND PNG

A. Participants from the Brisbane area (Queensland)

1. Department of Health, Queensland

Field : Epidemiology and Control of Malaria

Dr I.A. Musgrave, Senior Health Officer, is responsible for Department of Health overview of malaria epidemiology in the State of Queensland involving appropriate action relating to notifications of malaria cases, arrangement of investigations or other action to prevent transmission, and collaboration with the School of Public Health and Tropical Medicine in regard to providing information for the Central Malaria Register and obtaining blood films for the depository maintained by the School; also for advice to medical officers who are responsible for malaria patients, in regard to recent recommendations on treatment and investigation of patients.

This responsibility extends to an overview of the present Departmental procedures relating to maintenance of the malaria eradication status in Queensland which includes annual spraying operations in the Torres Strait, local spraying in response to a case, adequate treatment of cases and rapid investigation of outbreaks. Essentially, the latter is an overview of the passive case detection operation conducted under the local supervision of Dr Peter Holt the senior Medical Officer in the Torres Strait region. More recently, these activities have extended to the co-operative planning of a reconnaissance of the Torres Strait area by members of the 1st Army Malaria Research Unit with a view to possible involvement of army personnel in field operations in that area.

Dr R.B. Hawes, Health Officer (Aboriginal Health), provides assistance to the Senior Health Officer and relief when he is away in respect of supervision of malaria eradication activities described above.

Mr R. Luke, Microbiologist, Aboriginal Health Programme, is involved in malaria blood film microscopy during screening surveys and also entomological surveys in the Torres Strait.

2. Queensland Institute of Medical Research

Malaria Program

Personnel: Dr Chev Kidson (Lab. head), Ms Gretel Lamont (WHO), Dr Jackie Upcroft (NHMRC) , Mr P. Myler (Ph.D. Student), Dr Daniel Castelino (NHMRC Scholar), Dr Allan Saul (P/T), Mr Phil Chen (P/T).

Location: Queensland Institute of Medical Research, Branston Terrace, Brisbane, 4406

Grants: a. WHO grant for work on erythrocyte mutants in relation to malaria parasite receptors.

/b. NHMRC

b. NHMRC grant for work on the molecular biology of *Plasmodium falciparum* in relation to cloning of genes for protective antigens.

Objectives: a. To accumulate and characterize a library of Papua New Guinea strains of *P. falciparum* in long-term culture for general use. Currently the library has seven long-term cultured strains which have been characterized with respect to sensitivity to chloroquine and pyrimethamine.

b. To analyse erythrocyte mutants from Papua New Guinea which convey resistance against malaria parasites. The major objective is to identify membrane mutants and to this end studies have concentrated on malaria-resistant ovalocytes. These have been found to be resistant to merozoite penetration in culture. Current studies are dissecting their membrane chemistry to identify the mutant determinant.

c. To analyze merozoite antigens by monoclonal antibody techniques using merozoite invasion assays. A small library of such antibodies is already established and includes antibodies which inhibit merozoite invasion by binding to the parasites or by binding to erythrocyte surface antigens.

d. To carry our parallel work with mouse malaria species leading to specific merozoite antigen vaccination models. Work in this direction is well under way with *P. berghei*.

e. To identify protective merozoite antigens and to clone the genes coding for these. Basic work in this direction has commenced.

f. To examine mechanisms of drug resistance in order to define predictive indices. These studies are using Papua New Guinean and Thai parasite strains.

g. In collaboration with groups in Thailand to examine the feasibility of defining variant antigens as genetic markers for parasite population and drug resistance studies.

3. Royal Brisbane Hospital, Pathology Department

Dr Robin Cooke - Field : Diagnosis of Malaria

For some years now I have been involved in the treating of patients with malaria in the Royal Brisbane Hospital. I have also been giving the formal lectures on malaria to the medical students as part of their social and preventive medicine curriculum. In January 1979 it became obvious that the number of cases of malaria had increased dramatically. I called a meeting of the

/haematology

haematology staffs of the various laboratories in Brisbane to alert them to this happening and to arrange for a record of cases to be kept. The treatment protocol instituted by Professor Black at the School of Public Health and Tropical Medicine in Sydney was made available to all the laboratories, so that when doctors enquired as to the correct method of treatment, the laboratories were able to offer up to date advice. We were also able to collect clinical information by making enquiries of the referring doctor and sometimes of the patient as well, at the time when they had the infection. Follow-up information was also obtained from the doctors, partly as voluntary communications and partly as a result of retrospective enquiries.

At the end of 1979 a 5th year medical student, Miss Jenny Shannon, collected all the data and this has revealed quite a few interesting clinical problems.

a. People taking chloroquine prophylaxis developed *P. falciparum* infection which gave symptoms of "flu", "Dengue" etc that went on for weeks or months. These patients did not realise they had malaria, the symptoms did not suggest malaria to the doctors, and parasites were scarce in the peripheral blood.

b. If one member of a mixed-marriage family has malaria, the whole family should be tested, because in our experience other members of the family will probably have malaria, too.

c. Papuans and New Guineans have a high incidence of G6PD deficiency, and this should be tested before giving primaquine.

d. There was one case of cerebral malaria - the second one I have seen in 12 years at Royal Brisbane Hospital.

e. There were 2 cases of neonatal *P. vivax* malaria in children born at the Royal Women's Hospital in 1978.

f. In February 1980 a 38 year old woman presented a *P. falciparum* infection, almost certainly acquired by blood transfusion in Port Moresby in December 1979.

g. Malaria is still important for members of the defence forces as shown by 4 RAAF cases during the Kangaroo III Exercise.

h. Malaria is important in refugees - Vietnamese - mostly *P. vivax*.

i. There is a need to instruct laboratory workers in correct species identification.

j. In our society malaria is mainly a disease of the affluent. As a result the majority of patients are treated by the private sector. Treatment in both private practice and in hospitals was variable. It seemed to be more uniform

/among those

among those advised by pathologists. More education is needed.

B. Participants from the Canberra area (ACT)

1. John Curtin School of Medical Research, A.N.U.

Department of Microbiology

Principal Workers: Ian Clark*, Christine Rzepczyk, Paul Wood.

Field: The Role of Endotoxins in:

a. Illness and pathology of malaria.

b. Non-specific protection against haemoprotozoa.

Parasite Species: *Plasmodium berghei* (k173)
Plasmodium yoelii (17x)
Plasmodium vinckei petteri
(virulent and avirulent strains)
Plasmodium vinckei vinckei
Babesia rodhaini (Antwerp)
Babesia microti (Kings)

The following paper summarizes our programme of research.

A Model for Acute Malaria
(I.A. Clark)

This approach to malaria began by trying to understand why mice which had been injected with BCG, *C. parvum* and other agents with a prophylactic action against tumours are particularly good at withstanding infection with blood forms of certain haemoprotozoan parasites (1-6). Some other characteristics of this type of protection are the death of large numbers of parasites inside circulating red cells (crisis forms) and the apparent irrelevance of circulating antibody (2,3).

It has been suggested (7) that the events leading to these "crisis forms", and also much of the pathology of acute malaria and babesiosis, may be triggered by the release of something functionally like bacterial lipopolysaccharide (LPS) from the parasitized red cells at schizogony. This is of course much like the "malarial toxin" story of old, but with, in my view, several new twists. The main one of these is that the "toxin" is not directly responsible for harmful effect itself, but releases mediators harmful to the host (and perhaps also to the parasite) from macrophages.

* Was unable to attend the meeting, and therefore unable to discuss and answer questions at the meeting on his work. For this reason the paper he submitted was more detailed than others and includes references.

This model may be summarized as follows. At least some, and probably all, of these protective agents (BCG, *C. parvum*, etc) cause an outflow of new cells of the monocyte-macrophage series from the bone marrow (8). All of these agents increase the sensitivity of mice to the harmful effects of LPS. This increased sensitivity probably resides in the sensitivity of these new macrophages to LPS (9). When a small dose of LPS is given to these hyper-reactive mice a range of soluble factors, the mediators of endotoxin shock, soon appear in their plasma. Some of these mediators have been produced in vitro with macrophages from such mice. Both the outflow of new macrophages (10) and the accompanying increase in sensitivity to LPS (7) are also seen in mice with malarial infections. This model for the understanding of the processes of acute malaria predicts that something functionally similar to LPS, on release from parasitized red cells at schizogony, acts on these new LPS-sensitive macrophages to release these same mediators of endotoxin shock and that these mediators cause much of the illness we recognise as malaria.

The chemistry of some of these mediators, such as the prostaglandins, is well defined. There is evidence that the fever, haemodynamic changes and diarrhoea seen in endotoxin shock may follow release of these compounds (summarized in ref. 11). Other mediators of endotoxin shock are still known by the function by which they were first detected: Tumour Necrosis Factor (TNF) causes necrosis of certain established tumours, and their cytostasis or death in vitro (12,13), whereas Glucocorticoid Antagonizing Factor (GAF) antagonizes the ability of gluconeogenesis (14,15).

Not surprisingly this can drastically lower core temperature, a feature of algid malaria. GAF also exerts a cytostatic effect on hepatoma cells in vitro (16) and bone marrow cells in vivo (Berry, personal communication), and is probably identical to TNF. In theory this factor could harm malaria parasites, not just by analogy with its effect on tumour cells, but also because one of the enzymes whose induction it blocks may be important for the normal multiplication of malaria parasites. This may be the origin of "crisis forms". Chris Bryant will elaborate on this aspect during his comments.

We have also noted (17) that GAF could contribute to the liver damage seen in malaria. Curtailment of the supply of glucose for anaerobic glycolysis, on which the renal medulla depends (18), could also contribute to the acute degenerative changes in renal tubule cells. Clearly the hypoglycaemia and leucopoenia of acute malaria can also be explained by the actions by TNF/GAF. Earlier workers spoke of the similarity between certain forms of acute malaria and adrenal insufficiency, and tried, unsuccessfully on the whole, to explain this in terms of necrotic lesions within the adrenals (summarized in ref. 19). Their observations are consistent with glucocorticoid hormones being released normally, but not being able to induce gluconeogenic enzymes because of the presence of GAF.

Another mediator released when LPS is given to mice made hyper-reactive by prior infection with BCG is Migration Inhibition Factor (MIF) (20), which retards the movement of macrophages in vitro.

/There is

There is recent evidence (21) that the basis of this retardation is fibrin deposition on the macrophage cell surface - i.e. macrophages appear to release tissue thromboplastin after incubation with MIF. Thus some degree of fibrin cross-linkage and consequent consumptive coagulopathy, as reported in acute malaria, may result whenever MIF is released systemically. This has not yet been established in malarial mice, either terminally or when given LPS, though the blood clots poorly in each case. It should be noted that MIF has been demonstrated by inoculating cells from *P. berghei* infected mice with sonicated parasites (22).

Were these clinical and pathological aspects of malaria caused by mediators whose release was triggered by something of parasite origin which was functionally like LPS, one would expect a correlation between susceptibility to LPS and onset of illness. This appears to be so: host species such as man, which become ill at very low parasite densities on first exposure to malarial parasites, have a very much higher innate susceptibility to the harmful effects of LPS than do monkeys, rodents or birds. These species show no adverse effects from malarial infections until what in human terms are enormously high parasitaemias are reached (7). Thus one can infer that these individuals would have had SAA-inducer in their plasma. These authors note similarities in the circumstances and kinetics of production of SAA-inducer, TNF and GAF (26).

In summary, this model of acute malaria offers a unified approach to many clinical and pathological aspects of malaria, the origin of crisis forms, and certain field observations concerned with parasite densities and the onset of illness. Much of it appears to be testable.

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2. Department of Zoology, A.N.U.

Dr M.J. Howell

Parasites:

Blood stages of:

a. *Plasmodium berghei* - ex University of Queensland.

b. *P. yoelii* 17 x - ex Edinburgh.

Field: sex-hormones, immunization, auto-antibodies.

Current Research:

Two students (D. Reed and M. Agostino) have recently commenced projects on rodent malaria concerned with (i) the effects of sex hormones on parasite multiplication and (ii) vaccination. In relation to (i), nearly all previous work predates 1960, and there have been a number of conflicting observations. The second project is attempting to repeat some interesting, but largely ignored, work by D'Antonio (1972 Exp. Parasit. 31, 82-87) in which the injection of heat-killed blood stages of *P. berghei* into mice induced an impressive degree of resistance to challenge infection.

Recently completed work (M.J. Howell) has shown that the titre of autoantibody directed at antigen on enzyme treated red cells, which is low in the serum of normal mice, becomes elevated in mice

/with drug-

with drug-controlled infections of *P. berghei*. Similar elevations can be induced in normal mice by an injection of bacterial lipopolysaccharide. Such autoantibody does not appear to cause anaemia or to hasten the removal of infected red cells from the circulation.

Dr C. Bryant

(Other members of group: Mrs D. Bennett, Mr C. Budge, Ms R. Gauci, Ms K. Hare)

Field: enzymology.

Research

The malaria parasites share with some helminth parasites the property of 'homolactate' fermentation, yet they possess an active phosphoenolpyruvate carboxykinase (PEPCK) which has a high affinity for GDP and thus appears to function in the direction of CO_2fixation.

In other parasites, PEPCK is involved in energy metabolism, but in the 'homolactate' fermenters it must have another function. Gluconeogenesis can be excluded, as no known parasite is capable of it. We are therefore pursuing the possibility that PEPCK activity in the malarial parasite is related to purine nucleotide metabolism.

Our interest in PEPCK has led to collaboration with Dr I.A. Clark, also of the A.N.U. Zymosan sensitised, LPS challenged mice show suppression of gluconeogenesis caused by the failure of PEPCK induction by cortisone. This is mediated by GAF (glucocorticoid antagonising factor) and it is likely that *Coxiella* sensitised mice respond similarly. Dr Clark has found that sensitised mice infected with malaria do not inevitably succumb; instead, the parasite dies. Malaria infected mice also show signs of failure of gluconeogenesis. We are endeavouring to establish whether these effects are mediated by factors released from macrophages and, if so, what is the biochemical nature of their impact on the parasite. Mr C. Budge has just started a series of experiments designed to isolate macrophage factors produced by mice with malaria; Ms R. Gauci is attempting to characterise a component from isolated, sonicated parasites which, when injected into sensitised mice, produces an endotoxin-like shock; and Ms K. Hare is investigating the effects of serum fractions from parasitised mice on isolated parasites, whose viability is then tested by reinjection into mice.

Facilities

For malaria strains (*Plasmodium vinckei petteri*) and models, see Dr Clark's submission. The Zoology Department provides a well equipped laboratory for the maintenance and biochemical investigation of parasites.

3. Department of Human Biology, A.N.U.

Dr Philip Board, Mr K.C. Chockkalingam

Field: red cell metabolism.

/Our group

Our group has a substantial interest in red cell metabolism. It has previously been suggested that some red cell abnormalities may confer a relative resistance to malarial attack.

We are currently investigating glucose-6-phosphate dehydrogenase (G6PD) deficiency and hereditary ovalocytosis in Papua New Guinea (PNG).

G6PD deficiency is common in the coastal areas of PNG and is highly variable. So far eleven distinct variants have been identified.

Hereditary ovalocytosis is also commonly found in the coastal regions of PNG and is distinct from the forms of elliptocytosis occasionally seen in Europeans. This abnormality appears to be the result of a membrane defect which gives rise to antigen depression, abnormalities in membrane function and secondary effects on cellular metabolism.

We would appreciate receiving G6PD deficient samples from different areas of PNG.

4. Virus Ecology Research Group, Research School of Biological Sciences, A.N.U.

Dr Rod Mahon

Field: entomology.

Research Field: Identification of the biological entities within Australian *Anopheles* and in particular, *A. farauti* s.l. using polytene chromosomal analysis, cross mating experiments, electrophoretic techniques and multivariate analysis of morphological data.

Colonies: We have available *A. farauti* No. 1 and No. 2 recently derived from the field. At the moment they are not mating in cages, but must be maintained by induced mating.

Collaboration: We know nothing about the ecology, host preference, susceptibility to infection, etc of the biological species within the taxon *A. farauti*. Every assistance will be given to anyone interested in assessing any of the above. Live, wild-caught *A. farauti* would be appreciated at any time to add to our scanty distribution knowledge of the species within Australia.

C. Participant from the Darwin Area (N.T.)

1. NT Health Department, Environmental Health Division, Entomology Section.

Mr Peter Ian Whelan.
Collaborators: G. Davis, K. Holden, G. Williams.

Field: entomology.

Facilities Available: General entomology laboratory with normal laboratory equipment including microscopes, cold table sorting

/facilities,

facilities, liquid nitrogen containers, insecticide resistance kits, makeshift insectary, mobile caravan laboratory, two 4 wheel drive vehicles, CO_2 and light traps, mosquito truck trap. Reference collection of NT mosquitoes.

Research pursued:

a. Adult and larval mosquito monitoring around major population centres.

b. Collection of mosquitoes for virus isolation in southern laboratories.

c. Insecticide resistance testing.

d. Collection of *Anopheles* with SPH and TM for age grading determination.

e. Collection of *Anopheles* for ANU chromosome studies.

f. Mosquito distribution in the NT.

g. Biting and activity times of mosquitoes.

h. Effectiveness of mosquito buffer zones.

i. Assessing and advising on vector control operations in the NT.

j. Entomological investigations around imported malaria cases.

D. Participants from the Melbourne area (Victoria)

1. Walter and Eliza Hall Institute of Medical Research, Laboratory of Immunoparasitology

Field: Immunology.

Malaria/Babesia Programme

Current Personnel: G.F. Mitchell (Lab Head), R.F. Anders (Biochemist), G.V. Brown (Clinician), L.P. Kahl (Ph D. student), P.M. Smith (Bio-chemistry technician), R.J. Grumont (Malaria cultivation technician), and K.M. Cruise (Hybridoma technician).

Associates: PNG - Alpers, Stace, Vrbova, Heywood
Wacol - Callow, Rodwell, Mellors

Parasite Material: *P. berghei, p. yoelii, B. rodhaini* in mice. PNG isolates of *P. falciparum* in long-term culture. *B. bovis* blood from Wacol plus short-term culture.

Project headings (1976-79)

a. Changes in sialoglycoproteins on the surfaces of red cells

/from mice

from mice infected with lethal hemoprotozoa - Howard.

b. Isolation of parasitized erythrocytes from *P. berghei*, *B. rodhaini*, *P. falciparum* and *B. bovis*-infected blood using the FACS and according to DNA content of the cell - Howard, Battye, Brown & Wacol associates.

c. Production of host-protective antisera against *P. berghei*, in mice - Mitchell.

d. Immunoprecipitation analysis of biosynthetically-labelled *P. berghei* proteins using host-protective antisera - Knopf, Brown & Anders.

e. Changes in cell surface proteins and glycoproteins of bovine red cells infected with *B. bovis* and analysis of bovine red cell recognition by babesia parasites - Howard, Anders, Kahl and Wacol associates.

f. Inhibition of growth of *P. falciparum* in vitro using sera from clinically defined patient groups - Brown and PNG associates.

g. Immunoprecipitation analysis of biosynthetically-labelled *P: falciparum* proteins using sera from clinically-defined patient groups - Brown, Anders and PNG associates.

h. Production of hybridoma antibodies with in vitro inhibitory activity or of potential immunodiagnostic value and with specificity for human erythrocytes, soluble products of infected cells or schizont antigens - Anders & Brown.

Overall objectives of the research programme are to analyse host immune responses to defined antigens of human falciparum malaria in PNG population groups and bovine babesios : in Queensland cattle with a view to identifying host protective immunities, candidate vaccine molecules and possible immunodiagnostic strategies. A most pleasing aspect of the programme has been the establishment of productive linkages with colleagues in Australia and PNG and no aspect of the programme could have been developed without the skills, industry and generosity of these colleagues.

2. Fairfield Hospital

Dr Peter Cavanagh, Director of Laboratory Services,

Field: Malaria diagnosis.

Responsible for the laboratory surveillance of approximately 50 cases of malaria a year in returning travellers.

Collaborates by providing specimens for Dr Mitchell of the Walter and Eliza Hall Institute.

Is attempting to initiate drug sensitivity testing of strains of *P. falciparum*.

/3. Victorian

3. Victorian College of Pharmacy

Dr Peter Andrews*

Field: New anti-malarials.

We are presently planning a research programme on the design and structure activity relationships of antimalarial drugs.

The objectives of the project are:

a. Rational design of new antimalarial drugs exploiting known differences in tetrahydrofolate metabolsim between host and parasite. Initial emphasis will be placed on design of transition state analogue inhibitors for dihydropteroate synthetase and dihydrofolate reductase.

b. Computer assisted development of structure activity relationship for existing drug classes whose mode of action involve tetra-hydrofolate metabolism, viz. 2,4, diamino-pyrimidines, sulphones.

Resources: Over the past few years my laboratory has been involved in the development of several new techniques in the fields of structure activity relationships and drug design. Although we have no direct experience in the field of malaria research I believe that our drug design programme has now reached the stage where it could very fruitfully be applied to the chemotherapy of malaria.

Collaborative Projects: Our research would benefit greatly from collaboration with groups involved in:

a. Culture of parasites.

b. In vitro and in vivo testing for antimalarial activity.

c. Preparation and kinetic studies on enzymes in the tetra-hydrofolate pathway.

E. Participants from the Newcastle Area (NSW)

1. Faculty of Medicine, University of Newcastle

Robert Clancy, Allan Cripps, Greg Tannock

Field: Immunology.

Merozoite Penetration Inhibition

Aim:

a. To establish long term culture of *P. falciparum*.

b. To develop a microculture assay to detect inhibition of

* Was unable to attend the meeting.

red cell penetration by merozoites.

c. To use this assay, in conjunction with part-immune antisera, to define strain variants isolated in Madang, PNG.

d. To translate technology developed in Newcastle to the laboratories of PNG Institute of Medical Research in Madang, to type primary isolates in Madang.

e. To provide a seroepidemiological back up to the population/prophylactic study on Madang children, using indirect immuno-fluorescence.

We represent one component of the Madang based group co-ordinated by the PNG Institute of Medical Research, directed by Dr M. Alpers. The long term aim of this programme is to develop immunoprophylaxis in malaria. The primary aim of our current studies is to determine strain variation in the Madang area using a microassay. Initial studies will use culture-adapted *P. falciparum*. When optimal conditions are established and panels of partially immune antisera have been constructed, the assay will be developed in Madang to serotype primary isolates.

Once established this assay system can be used to ask a number of basic and applied questions related to the immunopathology of malaria.

In addition to the above, we will provide a "service" function to the group by (i) providing a further laboratory for the establishment of long term cultures, and (ii) providing a seroepidemiological assay to monitor the Madang chemoprophylaxis programme to examine one index of herd immunity.

F. Participants from Papua New Guinea

1. PNG Institute of Medical Research

Michael Alpers, Peter Heywood, George Nurse, John Stace and Helena Vrbova.

Fields: epidemiology, drug prophylaxis, immunology.

The Papua New Guinea Institute of Medical Research has instituted an integrated program of research on malaria in Papua New Guinea, to be carried out principally in the Madang Province, in collaboration with colleagues in Melbourne, Canberra, Sydney, Newcastle and Brisbane (who are presenting details of their own projects separately).

The program will have an epidemiological base. Regular demographic data will be collected on the study population, together with malariometric and haematological surveys and monitoring of morbidity and mortality. A pilot intervention program is already underway in a number of communities within the study area, involving the distribution of amodiaquine to young children through village dispensers. The effect of this program on health, parasitaemia rates and malarial immunity will be evaluated. At the same time studies on the host population will include an

examination of nutrition and genetic polymorphisms, with particular emphasis on those known to be associated with resistance to malaria. Non-malarial morbidity associated with certain phenotypes, and the response to primaquine, will also be investigated. Since a 4-aminoquinoline is being used for prophylaxis, the development of drug resistance by *Plasmodium falciparum* in the study area will be monitored. Associated entomological studies are planned.

The immune response to plasmodial parasites will be investigated in collaborating laboratories, with particular emphasis on dissecting the parasitic antigens and relating them to a range of specific immune responses in variously immune subjects, in the search for a measure of protective immunity.

Antigenic variation in the parasites will be studied. Fundamental to all such investigations is the need to establish in vitro cultures of malarial parasites from the area under study. At the same time, as methods for detecting protective immunity are being refined they will be applied to monitor the effect of the chemoprophylaxis program on the development of immunity in the population. Other leads may be obtained from a study of the inappropriate immune response to malaria as found in the tropical splenomegaly syndrome.

It is proposed to recruit village populations to the study by a slow process of expansion. At any given time some villages will be part of the intervention program and others will act as controls. Villages will be recruited first as controls and will later pass into the test group, during which time they will be under close supervision and surveillance. Finally, they will pass into the consolidation phase, when their capacity to maintain their own intervention program, and the long-term effects of this program will be under scrutiny.

The major aim of the research study is to investigate an alternative to vector control as a means of controlling malaria. The spraying program in the areas under study has not been successful and has been discontinued. Initially, the method being evaluated is a chemoprophylactic one. Ultimately, immunological studies here and elsewhere may lead to the development of a malarial vaccine. If this is finally achieved we aim to be suitably prepared to make best use of it, both immunologically, by ensuring that the vaccine produced is appropriate for use in Papua New Guinea, and epidemiologically, by having well-defined communities available in which to evaluate its effectiveness.

2. Laingam Rural Health Centre

Dr Peter Sharp

Field: Malaria in a rural health centre in PNG.

Malaria is a problem in low altitude valleys (below 1600 metres) of Enga Province, an area which is remote, rugged, and lacks basic health facilities.

There is no malaria control program in Enga Province and in the absence of treatment facilities and shortage of basic drugs,

recurrent epidemics of falciparum malaria cause high mortality.

People live with malaria, suffer, and die, much as they always have done.

So, this is also a reality in malaria over large parts of rural areas, where management is at least as important as research in the struggle for improving the state of those people.

G. Participants from the Sydney area (NSW)

1. Department of Tropical Medicine, School of Public Health and Tropical Medicine. (Commonwealth Institute of Health from 3.3.1980), The University of Sydney

Professor Robert H. Black

Malaria vigilance - epidemiological studies

The building up of a surveillance system has permitted the study of many aspects of the epidemiology of malaria in Australia over the past 11 years. This has been a study of what is possible in a cooperative venture within the Australian health system.

Some 2284 cases of malaria have been studied over the period 1969-79 from an epidemiological point of view so that we have a very good idea of the sorts of people who get malaria, the type of malaria they get and where they get it. Further, it has been possible to pick up changes in the malaria situation in other countries. This sort of information has immediate relevance to the advice given to travellers regarding chemoprophylaxis.

Again it has been possible to monitor the performance of medical practitioners in the clinical diagnosis of malaria and the capability of pathologists to make a correct microscopic diagnosis. As a result of this, attempts have been made to improve performance.

In the malaria receptive area the important application of this sort of work is the prevention of re-establishment of malaria.

Aspects of Malaria and Babesia Immunology

Work carried out in 1979 by Mr T. White in Department of Tropical Medicine who unfortunately left a few days ago for Perth where he will continue work on similar lines with Dr Thompson at Murdock University. He studied various aspects of the *P. berghei* model including some investigations based on the work of Clark.

Proposed research post in Torres Strait (SPHTM)

To investigate epidemiology of malaria in the area - hopefully including adjacent area of Papua New Guinea, act as a back-up for local malaria activities in the area, act as a field training centre for research and operations. Overall malariology with relevant components including entomology, parasitology, sociology. Associated with Queensland Health Department, 1st Army Malaria Research Unit, PNG Malaria Programme.

Affiliation of 1 MRU and Department of Tropical Medicine

Students for higher degrees (MSc, MPH, PhD) may now work at 1 MRU and be regarded as being "on campus" from the point of view of supervision.

Dr D.L. Houghton (Formerly Regional Malariologist, Papua New Guinea)

Malaria in the Papua New Guinea Highlands

Descriptive studies of work carried out during 2 years, 1978/79, in 4 provinces of the Papua New Guinea Highlands.

Spraying operations commenced in the Highlands during the late 1960's and were successful in lowering parasite rate to less than 1% for 3 successive years, 1971 to 1973. Prior to the commencement of malaria control operations malaria had been hypoendemic in the region, but unstable with seasonal and cyclic epidemics.

Spraying operations were withdrawn and it was proposed that a limited surveillance system should be established to monitor levels of malaria in the region and, through their early detection, to control small focal outbreaks which might arise. Full epidemiological investigation should be conducted around each positive case detected by passive case detection and limited spraying re-introduced together with mass drug administration in areas of malaria transmission.

The components of surveillance were:

a. a monitoring system which was devised as a co-operative programme between the General Health Services and the Malarial Control Programme to screen and administer presumptive treatment to every fever case presenting to a health post. All positive cases subsequently received a course of radical treatment and full epidemiological investigation (parasitological and entomological) was conducted around each indigenous case. A recording system was established to enable rapid detection of new areas of transmission.

b. a control system consisting of focal spraying operations (extended as required), single dose mass drug administration and health education repeated each 6 months. Limited chemoprophylaxis programmes were also commenced on plantations, some mission establishments and in areas where population density and rugged terrain prohibited spraying operations.

c. evaluation which was devised to provide ongoing analysis of the system as well as to monitor the effectiveness of control measures. The latter was made by parasitological and entomological surveys conducted prior to spraying and each subsequent cycle as well as by continuous monitoring of slide positivity rates from PCD returns in each locality.

/The surveillance

The surveillance system was established at a time of rapid socio-economic development in Papua New Guinea as a whole and the Highlands region in particular. Both vulnerability and receptivity to malaria increased with the development of communications, especially the construction of the Highlands Highway to the coast with access and feeder roads to almost every part of the region. Simultaneously, local population mobility increased with the breakdown of cultural mores accompanying social changes and there was an expansion of the development of coffee and tea plantations and other agricultural projects. Furthermore, large-scale integrated rural development projects were commenced in several provinces with the assistance of international agencies. The effects of socio-economic development upon the incidence and distribution of malaria were monitored through the surveillance system and comparison with the known history of the disease in the area. Control measures were adjusted and replanned according to the changing epidemiological situation.

Richard C. Russell, Medical Entomologist

Research Field (related to Malaria)

The major research project of the Entomology section is the development of the fungal pathogen *Culicinomyces* as a biological control agent. This is being undertaken in collaboration with the 1st Malaria Research Unit (Dr A.W. Sweeney) and is currently being supported by the World Health Organisation. Culture techniques, types of media, natural history of the pathogen, storage techniques and laboratory studies of its environmental limitations are among the avenues of research. I, personally, am undertaking population studies of potential target mosquito species (eg *Culex annulirostris*) with a view to devising a collaborative control model.

Another project of relevance to malaria is a study of the age structure of a population of *Anopheles farauti* from Darwin, NT. In collaboration with Peter Whelan of the NT Health Department the population is sampled fortnightly by CO_2/light traps (supplemented by seasonal visits for biting catches and resting catches). The catches are sent to SPH & TM for dissection and age-grading by the ovariolar dilatation technique. A laboratory investigation of the environmental requirements of the population, using eggs from wild caught females, is planned as part of the study in the hope that a life-table may be constructed for the "species" and its population dynamics better understood, possibly to the extent of constructing a population model for control purposes.

Facilities

Mosquito colonies:

a. *Anopheles hilli*

b. *Culex fatigans*

c. *Aedes aegypti*.

Mr John Walker, Medical Parasitologist

The major role of the medical parasitology sub-section in malaria research lies in the field of diagnosis of malaria. Blood films forwarded to the Central Register of Malaria Cases are cross-checked, providing data on the accuracy of species diagnosis of individual workers and other laboratories.

Facilities for malaria diagnosis, both by routine blood examination and fluorescence microscopy are provided.

The practical aspects of malaria diagnosis are taught to students in the School's degree and diploma courses and to individuals who wish to receive special training in malaria diagnosis.

2. School of Biochemistry, University of New South Wales

Professor W.J. O'Sullivan, Mrs A. Gerö (on leave in Glasgow), Mrs E. Hagan

Field: Malarial Enzymology

Research Area

Basic biochemistry of the metabolism of nucleic acid precursors, particularly de novo synthesis of pyrimidines. In the intra-erythrocytic stage the malarial parasite can obtain purines by salvage pathways from the pool in the erythrocyte. However, the pyrimidine pools are much smaller and the parasite must synthesize these compounds in order to replicate.

The intended emphasis is on the enzyme dihydroorotate dehydrogenase (DHO-DHase) which converts dihydroorotic acid to the first pyrimidine compound, orotic acid. This is a mitochondrial enzyme that is present in red cell precursors but is lost on maturation. Thus, this activity is unique to the parasitized cell with respect to the uninfected cell.

Work has been carried out on the dehydrogenase from human spleen mitochondria and more recently from *Plasmodium berghei* and mouse reticulocytes. It is hoped that this will be extended to *Plasmodium falciparum* in the near future. Our current aims may be summarised as follows:

a. To characterize pyrimidine metabolism in *P. falciparum*, with particular reference to a study of dihydroorotate dehydrogenase.

b. To study inhibition of the DHO-DHase (both pyrimidine analogs and respiratory chain inhibitors) as a basis for the design of potential antimalarials.

c. The possibility that assay of DHO-DHase could provide a biochemical assay for the degree of infection shall be investigated.

The malarial work is essentally embryonic at this stage. However, the laboratory has considerable expertise in pyrimidine and purine metabolism and, with a small grant from the NH & MRC and at least one PhD student devoted to the area, we expect to be a viable concern by the end of 1980.

/We have

We have no strains available in our laboratory.

In the first instance, we shall be very much dependent on being able to obtain material from laboratories with *P. falciparum* strains in long term culture. At later stages, we expect to be interested in blood samples from subjects with various levels of infection.

3. Division of Haematology, Repatriation General Hospital, Concord

Dr G.G. Crane, Director

Collaborators: Dr H.V. Bashir, Deputy Director, NSW Blood Transfusion Service, 153 Clarence Street, Sydney, NSW 2000

Dr M. Alpers, Director, Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, PNG.

Research Project

Background: Tropical splenomegaly syndrome is one of the two major clinical entities arising from atypical immune responses to malarial infection. It is an uncommon manifestation of recurrent malaria in most endemic countries, but in Papua New Guinea it affects up to 80% of some tribal groups, and is a major cause of morbidity and mortality.

Extensive field and hospital studies in Papua New Guinea have failed to reveal an abnormal pattern of malarial infection, or any additional aetiological agent. Immunity to malaria, as judged by parasite rates at different ages, develops normally in affected subjects, indicating that production of protective malarial antibody is probably unimpaired. Serum IgM levels, however, are disproportionately elevated even in young children prior to the acquisition of significant immunity, when their splenomegaly is clinically indistinguishable from simple malarious splenomegaly. IgM levels continue to rise markedly throughout life in those in whom the syndrome develops.

Much of this IgM is immunofluorescent malarial antibody (IFA) though auto-antibodies such as rheumatoid factor and cold agglutinins also contribute. The IgM takes part in the formation of high molecular weight cold-precipitable immune complexes (which may also contain IgG and complement). No antigen has been characterized in these complexes, though IgG and IgM IFA activity has been detected. Trappings of immune complexes appears to be the cause of the characteristic hepatic sinusoidal lymphocytosis, and of the prepetuation of the splenomegaly. They may also be responsible for the acute haemolytic episodes which characterize the disease.

Long term administration of prophylactic antimalarial drugs produces reversal of all manifestations of the syndrome in most subjects, though complete regression of gross splenomegaly may take years to achieve.

/Present research

Present research programme

The fundamental defect has yet to be elucidated, but epidemiological data point to a genetic basis. Current investigations are centred on the inhabitants of the Upper Watut Valley, where most of our field studies have been carried out since 1964. They relate mainly to:

a. continuation and extension of a programme of administration of antimalarials on a village basis, which has been in operation since 1971;

b. evaluation of the effects of residual insecticide spraying (commenced in 1978) on the prevalence and severity of the syndrome in previously unprotected villagers;

c. investigation of the genetic basis of the syndrome, particularly of any association with HLA antigens;

d. characterization of the disturbance of immune responsiveness preceding the development of the overt syndrome, particularly relating to abnormalities in antimalarial antibodies (class, specificity and affinity) and of immune complex formation;

e. the serological basis of the anaemia, and particularly of the acute haemolytic episodes which punctuate the course of the disease.

As will be apparent from the above outline, this is a collaborative project employing the facilities of the 3 institutions listed, and in addition involving in specific areas other members of the PNG/Australian malaria research group - in particular the Queensland Institute of Medical Research, The Walter and Eliza Hall Institute, and the Department of Immunology, University of Newcastle.

4. Royal Australian Army Medical Corps

First Malaria Research Unit (1 MRU)

Director: Dr G. Gramiccia

Deputy Director: LTCOL A.D. Parkinson, Research Officer, Parasitology

Research Officer, Entomology: MAJ A.W. Sweeney

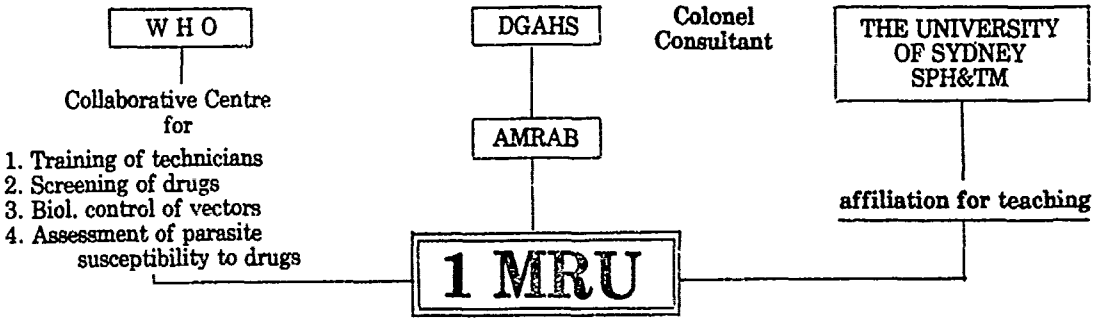
Research Officer, Biochemistry: CAPT M.D. Edstein.

/Present Positions

POSITION AND FUNCTIONS OF MRU

In December 1979, the Malaria Research Unit (MRU) was affiliated to the School of Public Health and Tropical Medicine of the University of Sydney for teaching purposes. Post-graduate students can now be attached to MRU for their studies and research work. The recognition by the World Health Organisation (WHO) of MRU as collaborative centre in the field of training and research on malaria is in progress. The following diagram represents the present position of MRU and its functions in terms of services, research and maintenance of strains (malarial plasmodia, mosquitoes and fungi capable of controlling mosquitoes) for research purposes.

PRESENT POSITION AND FUNCTIONS OF 1MRU



SERVICES

1. Training of staff (technicians, graduate, post-grad.)
2. Testing resistance to drugs of human plasmodial infections
3. Epidemiological surveys
4. Provision of specimens/strains for training/research
5. Diagnosis and treatment of malaria cases
6. Co-ordination of research involving Armed Forces, civilian Institutions, WHO
7. Possibility of use of human volunteers

RESEARCH

1. Drug metabolism and related enzymes
2. Cultivation of *P.berghei*
3. Effect of drugs and combination of drugs on normal and resistant strains of *P.berghei.*
4. Biological control of mosquito larvae with *Culicinomyces* and other fungi.
5. Genetic speciation of local vectors of malaria.

MAINTENANCE OF STRAINS

1. *P.berghei* ANKA in albino mice
2. *P.berghei* resistant to 50 MED pyrimethamine
3. *P.berghei* resist. to 50 MED chloroquine
4. *P.berghei* resist. to 7 MED mefloquine
5. *An.farauti* n.1, Rabaul, PNG
6. *An.farauti* n.1, Port Moresby, PNG
7. *An.farauti* n.1, Gove, NT
8. *An.farauti* n.2, Bamaga, Q
9. *An.annulipes,* Sydney, NSW
10. *An.hilli,* Gove, NT
11. *C.fatigans,* Sydney, NSW
12. *Culicinomyces,* sp., Ingleburn, NSW
13. *Culicinomyces clavosporus,* N.Carolina, USA
14. *Smittium culisetae,* Sydney, NSW
15. *Smittium, n.sp.,* Sydney, NSW
16. *(Aotus trivirgatus*/human plasmodia, to be established shortly)

PAULATIM

/LTCOL A.D.

LTCOL A.D. Parkinson, Parasitology Section

The parasitology section maintains the following strains of *Plasmodium berghei* for the purpose of morphological, drug and metabolic studies and in vitro cultivation:

a. ANKA strain which at the present time is undergoing tests for recharacterisation.

b. A pyrimethamine resistant strain which is stable and resistant to 80 mg/kg pyrimethamine, ie 50 times the minimum effective dose.

c. A chloroquine resistant (50 MED) strain which is in the process of development but which appears to be stable after 30 passages.

d. A mefloquine resistant (8 MED) strain which is still in the process of development and has not yet been characterised or tested for stability.

Facilities are provided for the diagnosis and treatment of malaria and the in vitro testing of specimens of blood for drug resistant strains of human malaria. Normally the macro-method is used but the Unit has been developing its own micro-method plates, based on WHO criteria.

Epidemiological and operational surveys are conducted as required.

A programme is being developed for the utilisation of *Aotus* monkeys for the testing of strain stability and the effects of various drug combinations.

A study of baseline biochemical data in human volunteers is planned as a forerunner to studying the effects of certain anti-malarials singularly and in combination.

CAPT M.D. Edstein, Biochemistry

The section is principally involved in in vitro cultivation and chemotherapeutic studies of the rodent malaria, *Plasmodium berghei*.

The objective of in vitro cultivation is to develop a technique to the point where it can be used to determine the sensitivity and resistance of selected species to chemotherapeutic agents. The technique used is commonly referred to as the 'rocker-dilution system'. It involves diluting infected blood with the growth medium RPMI 1640 in a novel cultivation vessel. The vessel is placed in a water bath set at 38°C. The system is agitated and gassed continuously with 5% CO_2 in air.

Based on morphological criteria, *P. berghei* appears viable after 42 hours of cultivation. Efforts are being undertaken to improve on this result by demonstrating growth and multiplication of the parasite.

/The second

The second project currently being investigated is the pharmacokinetics of chloroquine in mice given 250 mg salt/Kg. Chloroquine levels are determined in plasma and tissues using a spectrofluorometric technique. The section is also capable of measuring dapsone and quinine fluorometrically and pyrimethamine by photometric procedure.

Comparison of the pharmacokinetics of two antifolate drug combinations, maloprim and fansidar will commence shortly. High performance liquid chromatography techniques will be used to monitor the levels of the antifolate drugs in plasma and tissues. These studies will be conducted using in vivo and in vitro applications.

Specialised Equipment - 1 MRU

Equipment	Description
Calculator	Hewlett Packcard HP9815A & 97 (Programmable)
Chromatography	Pye Unicam LC3 Modular system (High performance liquid) - arriving shortly
Chemistry Unit Auto	AC1 - sample diluter, reagent addition, Mixer, Incubation and transfer of processed solution - arriving shortly
Electrophoresis Kit	Gelman
Freezer	Biological, ultra low with liquid nitrogen backup
Incubator	a. "Gallenkamp" cooled model IH-760
	b. "Gallenkamp" cooled insulation
Inoculating	Laminar flow chamber (x2)
Pump	Peristaltic (x2)
Refrigerator	Serological
Spectrofluorometer	Farrand Model 801
Spectrophotometer	Pye Unicam SP8-100 UV/VIS
	Scanning accessories:
	a. Rate rack system
	b. Densitometer
	c. Autocell
	d. Liquid Chromatography kit.

/MAJ Tony

MAJ Tony Sweeney, Entomology Section

Current Research Activities

Microbial Control of Vectors

Research conducted at MRU during the last eight years in collaboration with the Entomology Section of the SPH & TM, the University of Sydney, has demonstrated that the fungus *Culicinomyces* is a promising candidate for the control of mosquito larvae: it can be mass-produced in fermentation culture; it is specific for mosquitoes and closely related aquatic dipterous larvae; and is apparently safe for vertebrates.

Other institutions are collaborating in various aspects of this study: the Electron Microscope Unit (on ultrastructure of fungus invasion in mosquito larvae) and the Department of Veterinary Clinical Studies, of the University of Sydney (on vertebrate safety testing); the Commonwealth Serum Laboratories, Melbourne (on industrial production); and the Department of Botany, University of North Carolina (on taxonomy).

The involvement of CSL, in production of the fungus on a semi-industrial scale, will enable field tests against natural populations of mosquito larvae. It is planned to conduct a trial (several hectares in area) at a suitable location in the Murray Valley, against the Australian encephalitis vector - *Culex annulirostris*, during November-December 1980.

Other microbial control research activities will be carried out in 1980 on the entomogenous bacteria *Bacillus sphaericus* and *Bacillus thuringiensis israelensis* against local species of mosquito larvae, in association with Dr Elizabeth Davidson of Arizona State University, USA.

Speciation of *An. farauti*

Several colonies of *Anopheles farauti* are maintained at MRU for genetic studies on this important malaria vector of the Region. Dr Rod Mahon, of the Vector Ecology Research Group, Australian National University, is collaborating in this endeavour. Initial efforts will be directed towards establishing the identity and distribution of the sibling species of farauti in Australia. This will complement similar work to be carried out in Papua New Guinea by Mr Paul Hudson, of PNG Malaria Service, and Professor Tozo Kanda of St Marianna University, Kawasaki, Japan.

Future Plans

Experimental Transmission of Malaria. After the arrival of *Aotus* monkeys in the unit, experimental transmission attempts of human malaria parasites will be conducted with the *Anopheles* colonies with a view to maintaining the sporogonic cycle on a regular basis.

/Facilities

Facilities

The facilities at the disposal of the entomology section of MRU include:

a well appointed insectary for maintenance of mosquito colonies, and

a laboratory and associated equipment for experimental manipulation of mosquitoes and entomogenous microorganisms.

5. Commonwealth Scientific Industrial Research Organisation, Division of Animal Health, McMaster Laboratory, Glebe.

Alan L. Dyce

Major Interest: Culicoides biting midges and arthropod transmission of arboviruses.

CSIRO is not involved in malaria research. Our experience in long term studies aimed towards preparedness to meet the entry into Australia of a range of exotic arthropod borne diseases of livestock has practical relevance to the potential problem of local malaria outbreaks.

Very little interest is being shown currently to the potential field vectors of malaria in Australia. It is suggested that, in the event of a local outbreak, effective suppression of field vector populations would be seriously hampered by a dearth of knowledge of basic bionomics, behaviour and population dynamics of those species relevant.

Such necessary reliable information to meet the real emergency is unlikely to be gathered via the common practice of sporadic excursions to the field. Continuous study at permanent field stations of a kind that would gauge cyclical changes is suggested as the most profitable approach. Vector studies are part only of the overall epidemiology and should be interelated with other relevant disciplines. However care must be taken to ensure continuity of the entomological objectives in any such porgramme.

6. The Australian Society for Parasitology

Dr J.C. Boray (Vice-President) and Dr P.D. Crowfoot (Honorary Secretary)

Research Area

Development of new anthelmintics, insecticides and acaricides for use in the treatment of animals and in general hygiene and pest control. A wide area is covered, from primary screening of new chemicals to registration trials. Special interests include all aspects of liver fluke biology and biochemical mode of action studies.

/Facilities

Facilities

Many of the common helminths, insects and ticks of sheep, cattle, dogs and cats are maintained at the Research Centre. Insects of stored grain and general hygiene pests such as cockroaches are also maintained in our laboratories. We are equipped to conduct drug residue assays in tissues and environmental samples by GLC and HPLC techniques, toxicological studies including clinical chemistry, and metabolic studies by spectrophotometric methods.

7. Wellcome Australia Limited

John H. Ardley, Senior Scientific Adviser - Entomology

Group Members include: J.P. Brooke, Wellcome Foundation Ltd., J.C. Wickham, Chief Entomologist, WFL, Berkhamsted, Dr P.R. Chadwick, Research Entomologist, WFL, Berkhamsted, Dr D.B.A. Hutchinson, Clinician, WFL, Beckenham, Kent.

Group Interests

a. Development of permethrin 25/75 (NRDC 143) and decamethrin (NRDC 161) for vector control programmes. Eg - J.P. Brooke - Mosquito News, 1976 36(4); 402-411 J.P. Brooke and J.B. King - 1977 Mosquito News 37(3); 439-443.

   Concept - ulv spraying to reduce anopheline (or man-biting vector) contact or culicine nuisance, in localised area of camp or village etc using LECO HD or MINI ULVA spinning cup machines to apply pyrethroids at <10g/hectare.

b. Development of pyrethroids at low dosages for surface residual spraying of hut interiors (Ref: WHO/VBC/78.701). Progress - recent Kenya researchers have shown permethrin wettable powder at 125 mg ai/m2 and decamethrin at 15 mg ai/m^2 both superior to fenitrothion at 2000 mg ai/m^2 in control of endophilic mosquitoes.

c. Development of permethrin as:

   (1) repellant on uniforms and socks - current UK army trials in tropics show tick repellency,

   (2) mothproofing agent, uniforms etc,

   (3) head and body lice control.

d. Development of permethrin at 25 mg ai/m^2 on mosquito netting in limiting penetration by *Ceratopogonidae* and *Simuliidae* - established benefits from UK army trials in central America.

e. Development of pyrethroids in consumer products, eg coils, vapourisers, smokes, aerosols, sprays etc.

f. Interest in progressing Australian, Papua New Guinea and

/Pacific Area

Pacific Area, development of pyrethroid insecticides.*

Facilities Available

a. Wellcome Research Laboratories, Berkhamsted, UK. Assessment of resistance in vector species. Participation in and assistance with field research in Public Health and vector control in agreed Research Programmes.

b. Wellcome Research Station, St. Mary's. 40 hectares given over to agreed research programmes.

H. World Health Organisation, Regional Office for the Western Pacific Region, Manila, Philippines

Dr Willem J.O.M. Van Dijk, Regional Malaria Adviser

Problems hampering progress of the antimalaria programme in the Western Pacific Region

Some years ago major obstacles to progress in national antimalaria programmes in so far as these could be removed through applied field research were identified in the WPR on the basis of information available at the Regional Office. These areas for research were summarized in WPR/MAL/30.

It seems of interest to mention that it appeared to be necessary to update this summary within two years (WPR/MAL/32), not so much because the original information had been incomplete, but due to the fact that some technical developments had opened new avenues.

A workshop for directors of the regional antimalaria programme held in Kuala Lumpur in September 1979 provided the opportunity to have a more complete picture of the problems faced, with, in addition, an indication of the importance attached by the respective programme directors to the problems in terms of hindering the programmes' progress. The outcome of this review is given in the attached table.

The identification of regional priorities on the basis of this review is not as easy as it appears to be initially:

a. While few member countries would object to giving priority to chloroquine resistance and related studies, the problem is of minor importance in China.

b. The problem of *A. sinensis* is only of interest to China, where it affects however millions of people in the central part of the country, and is responsible for the great majority of malaria cases still being experienced in that country. One may argue, on the other hand, that as only *P. vivax* is involved, the problem deserves a lower priority

c. Does *A. farauti* deserve less attention, since it has been mentioned as a major problem by two project directors only, than *A. balabacensis* which has been mentioned by five directors?

* bioresmethrin, tetramethrin, allethrin forte, bioallethrin, Esbiothrine, d-phenothrin, phenothrin, permethrin, decamethrin, ethanothionyrethrate and developmental compounds

d. The problem of G6PD deficiency in relation to the use of primaquine is listed by five of the eleven projects. The subject is not popular among malariologists, but the MEP in Peninsular Malaysia assigns highest priority to it, as it is a major obstacle on their way to ultimate eradication of malaria.

e. The review contains some important information for the CHEMAL SWG: five of the eleven projects are awaiting an improved methodology in the radical treatment of *P. vivax*; China and Solomon Islands give it high priority.

From the above, it is concluded that in setting priorities for applied field research at the regional level, the following factors should be taken into account:

a. The total population which is likely to benefit from the outcome of the research.

b. The degree of the benefits to be expected.

c. The number of countries likely to benefit.

d. The importance of the problem in hindering progress in an individual country/countries.

e. The potential applicability of the research outcome elsewhere.

It is appreciated that some of these points are conflicting. Since priorities are being set with the purpose of deciding on external support, one should perhaps add:

f. The capability of countries to fund researches themselves.

On the basis of these criteria, it is proposed to support with Priority A:

a. chloroquine resistance and related studies.

b. G6PD deficiency studies.

c. Radical treatment of *P. vivax* studies.

d. *A. balabacensis* studies (including alternative insecticides).

e. *A. farauti* studies (including alternative insecticides).

f. *A. sinensis* studies.

g. Community participation studies.

Priority B:

a. Studies on long incubation period of *P. vivax*.

b. *A. litoralis* studies.

c. Study side-effects of DDT in Papua New Guinea.

/d. Alternative

d. Alternative insecticides in the Philippines.

e. Alternative vector control measures in the Philipinnes.

f. Maintenance phase operations studies.

PRIORITY AREAS FOR APPLIED FIELD RESEARCH IN MALARIA IN COUNTRIES OF THE WESTERN PACIFIC REGION

earch orities ntified / Countries	Republic of Korea	Lao PDR	Peninsular Malaysia	Sabah Malaysia	Sarawak Malaysia	New Hebrides	Papua New Guinea	China	Philip-pines	Viet Nam	Solomon Islands	Countries Maintenan
blems related with *vivax* with long ubation period								X				
ical treatment *vivax*	X				X		X	XX			XX	
loroquine resistance l studies on alter-tive drugs		XX	X	XX	X		XX	X	X	XX	X	X
PD deficiency			XX	X				X		X	X	
blems related with *balabacensis*		X		XX				X	X	X		
farauti							XX				XX	
sinensis								XX				
litoralis									X			
udy DDT side-effects							X					
ternative insect-ides									X	X	XX	
ternative vector ntrol measures									X			
mmunity rticipation					X	X	XX		X			
intenance phase erations												XX

ANNEX C

LIST AND ADDRESSES OF PARTICIPANTS INVITED
(in alphabetical order)

The name of participants having attended the meeting is underlined. Participants whose name is preceded by an asterisk are the leaders of a group of research workers.

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