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CONTROL AND ERADICATION OF
PLASMODIUM SPP. IN MAN

by

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B.S., Kansas State Univ., 1973

A MASTER'S REPORT

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Parasitology

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1978

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ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to all faculty and students for their support. My special thanks to Dr. M. F. Hansen for his continued help and understanding.

I. Introduction

With the development of D.D.T. as an insecticidal tool during W.W. II, it was hoped that world-wide control of malaria could be achieved. Now some 30 odd years later, malaria still kills an estimated one million people a year, with an additional 50 million suffering from some aspect of its attack. Although great advances have been made, (in Africa north of the Sahara, 67 million people are under malaria risk, but 95.5% are protected by some type of anti malaria measure); in other areas, however, such as the Indian sub-continent, the number of people under malaria-risk has increased against a previously decreasing trend, by 300 million people (W.H.O. bulletin, 1977). This increase is possibly one due to a let down of effort, but can also be traced to the development of resistant strains of mosquitoes and Plasmodia. Undoubtedly, social and economic development of afflicted areas will be determined by the continuing success of malaria eradication.

If the appearance of malaria can be approximated by the origin of its vector, then it is an ancient disease indeed, as mosquito like fossils that are 100 million years old have been found. In addition, the development of resistance mechanisms in man, such as the sickling trait (which gives increased protection to heterozygotes until they can achieve immuno-competency) or the Duffy-blood groups, imply an extremely long association. Probably the first to record the associated symptomologies of the disease was Hippocrates (460-377 B.C.), who noted both the intermittent fever and the palpability of the enlarged spleen, which is still a diagnostic characteristic. However, our name for the disease is derived from the Italians, who described the disease as being due to mal (bad) - aria (air). Even then, an association between the disease and swampy areas was noted. It was not until after Alphonse Laveran, in 1880, demonstrated the intraerythrocytic parasites, that Patrick

Manson persuaded Ronald Ross, in 1894, to test the hypothesis of mosquito transmission.

Malaria is still generally associated with rural settings, and one could thus assume that with increasing urbanization, the problem would disappear. However, it should be remembered that mosquitoes can breed and reproduce in tremendous numbers in very limited environments. In fact, the tendency towards urbanization can actually precipitate an increase in malaria incidence, as the mosquitoes that would normally feed on the now displaced large vertebrates, turn to man as a food source, thus increasing their role as possible vectors. In addition, the construction of various projects (roads, railroads, dams, irrigation systems) that accompany the process of urbanization, tends to increase the available habitats of many vector species. It has also been found that the haphazard growth of shanty towns around growing urban centers without the benefit of proper sanitation and waste water disposal also increases available breeding grounds.

II. The Disease

Malaria, in man, can be caused by any one of four protozoan species; Plasmodium falciparum, which has a 6 day incubation period from inoculation to appearance in the blood, is asynchronous in its development of the erythrocyte invading cells (merozoites); P. vivax - an 8 day incubation and a synchronized merozoite release (48 hr. cycle); P. malariae - a 27-37 day incubation and a 72 hr cycle P. ovale - a nine day incubation and a 48 hr. cycle. Of these four, the first two account for approximately 95% of all cases worldwide. The species P. malariae is unevenly distributed throughout the world, and P. ovale is restricted to Africa south of the Sahara. The life cycle of Plasmodium spp. is split into an asexual phase (in man) and a sexual phase (in the obligate vector - some Anopheline mosquito). Beginning with the injection of the

sporozoite form (found in the saliva of the female Anopheline) into man, there is a rapid clearing of this stage from the blood (within a half hour), as they take up residence in the parenchyma cells of the liver. The selection of the liver as its first site of infection has been suggested (Peters, 1970) to be due to the high activity of the pentose phosphate shunt and other ATP producing systems of these cells (Plasmodium spp. like many other obligate intracellular parasites, have a "leaky" cell membrane and are able to freely transport large macromolecules, like ATP, across it), and to the high availability of such metabolites as folic acid derivatives, pyrimidines, and purines, all of which are necessary for the nucleic acid synthesis which occurs during this stage. After multiplication in the liver (termed schizogony), the cells lyse, releasing from 10,000 to 40,000 merozoites (depending on the species). These then invade erythrocytes, and transform into the trophozoite forms, which in turn develop into schizonts. These schizonts then develop into the characteristic number of merozoites, lyse the cell, and repeat the erythrocytic cycle. Eventually some merozoites will develop into gametocytes (immature gametes) of which there are two types - micro and macrogametocytes. Once mature, the gametocytes can be found circulating in the peripheral blood, where a feeding mosquito can pick them up. Once ingested in the female mosquitoes' midgut, exflagellation of the gametocytes occurs (gametogeny), resulting in mature micro- and macrogametes. These then fuse to form the zygote, which in turn develops into an ookinete. This form penetrates the brush border of the epithelial cell of the midgut by liquefying the cells' membrane (Herms, 1969); once through, it assumes a spherical shape and forms a cyst wall to become an oocyst. Following growth (including meiotic and mitotic divisions of the nucleus), the oocyst bursts, releasing the infective sporozoites into the hemocoel. Although the sporozoites

spread throughout the body, many eventually make their way to the salivary glands, where they penetrate and concentrate in the acinal cells. When the mosquito feeds, saliva is first injected into the wound carrying with it the sporozoites and thus the cycle is completed. Development in the mosquito is dependent on temperature. Below 16 degrees C., no development will occur. Likewise, at higher temperatures this phase can be speeded up. At 23 degrees C., it takes 12 and 16 days for P. vivax and P. falciparum, respectively, while at 28 degrees C., it is shortened to 8 and 11 days, respectively.

In all types of malaria, there are characteristic periods of fever and chilling. These are caused as a result of the rupturing of the erythrocytes (RBC's) by the merozoites, thus releasing pyrogens into the blood; these in turn trigger a heat conserving response in the host. These alternating periods are most obvious in non-falciparum types, as they have a generally synchronized release of merozoites. This lack of synchrony on the part of falciparum infections is partially responsible for its high mortality rate. Because the merozoites are released in a non-synchronous manner, it is possible for a falciparum infection to be well advanced before it becomes clinical. This, coupled with the general "stickiness" of RBC's infected with P. falciparum (which causes them to tend to clump, thereby resulting in localized blockage of blood to vital areas), can allow a great deal of damage to occur. In all types of malaria, decreasing numbers of RBC's can lead to anemia, with up to 1/5 of the RBC's being destroyed.

III. Eradication

In 1956, the World Health Organization made eradication a major priority, and offered the following definition:

"Malaria eradication means the ending of the transmission of malaria and the elimination of the reservoir of infective cases in a campaign

limited in time and carried to such a degree of perfection, that, when it comes to an end, there is no resumption of transmission". It should be noted that this does not call for the eradication of the Anopheline vector, only for removal of the disease. Although vector control is an important aspect of malaria control, it is possible to achieve malaria eradication without complete removal of Anophelism, a state that most public health authorities view as being unattainable on a worldwide scale. Programs aimed at the vector can be directed against either the larval state or the adult. Although a good larvicide program would seem to be invaluable, there are many difficulties, as noted by Pampana (1969). First, it is difficult to map or identify all breeding places in a given area. When that area is located in a place of high rainfall, the problems are multiplied greatly, as new breeding grounds may be created from week to week. Second, the larviciding cycle is short and occurs with such frequency that it might be necessary to treat breeding places every week or five days. Third, it is difficult to be certain that all breeding areas have been treated, especially if regions are inaccessible or uninhabited. Finally, a successful anti-larval campaign is expensive and could be prohibitively so for a poor or sparsely inhabited area. These considerations argue against the use of anti-larval campaigns, except in conjunction with house spraying and/or drug administration. However, in the wake of ecological concerns over the effect of residual chemicals, there has been an increasing effort to find and utilize biological systems of control. One of the more impressive successes has been achieved with the minnow-like fish, Gambusia affinis. Another possible candidate is the common guppy, Lebistes reticulatus. Both are voracious feeders on both the larval and pupal stages of mosquitoes.

The second target of vector control is, of course, the adult. As

suggested by Pampana (1969), there are four properties that an insecticide must have in order to be effective; first, it should be a contact insecticide that can kill within an hour or less. To this end, it should be composed of small particles that can readily be picked on contact; second, it should have good residual activity (last for three months after spraying); third, it should not be as toxic as some of the organo-phosphates (parathion) are to man; and finally, it should be of low cost, and, hopefully, should not have an objectional color or smell, as this might dissuade its use.

A possible spraying schedule for DDT would be twice yearly spraying at the rate of 2 gm/m^2 ; for dieldrin, twice yearly spraying at the rate of 0.6 gm/m^2 .

However, it should be kept in mind that if eradication of a vector species is successful, this will create an available ecological niche into which a previously unimportant secondary vector could move, and create new problems.

Besides such measures aimed at the vector, control measures can also be applied in the human host, often with better, longer lasting results. First, there is the avoidance method. By restricting movements during the times of heaviest feeding by the female Anopheline (approximately dawn and dusk), it is possible to reduce exposure to malaria transmission. Coupling this with the proper screening of houses (to prevent entry of endophillic species), or at least the use of mosquito netting at night, and application of repellent compounds such as N,N diethyl-m-toluamide or ethyl hexaneidiol, one should be able to lower ones vulnerability. However, such methods are impractical for many poor countries to implement. This necessitates the development and use of prophylactic and therapeutic drugs. For centuries, Peruvian Indians had been using the bark of the chinchona tree

as a means of both prophylaxis and cure. With the coming of the Europeans about 350 years ago, and especially after the extraction and purification of this febrifugal compound in 1820, quinine became the primary drug of choice. Quinine remained the most successful treatment until the recent development of the new anti-malarials, many of which are derivatives of the basic quinine structure, (e.g. the 4-aminoquinolines, of which chlornquine is the most effective and most widely used, and the 8-aminoquinolines). Even today, with the development of chloroquine-resistant strains, quinine is suggested as the first alternative treatment, (Pampana, 1969).

With the advancement of improved cell biology techniques, it has become possible to investigate the mode of action of many of these drugs. Carter and Van Dyke (1972), studied the effects of the drugs quanicrine and ethidium upon chloroquine sensitive and chloroquine resistant strains of P. berghei (a malarial parasite of mice). By measuring the amount of phosphorylation of adenosine and its incorporation into nucleic acids, the drugs could be seen to affect both phosphorylation and polymerization, but that the inhibition of polymerization occurred at such a lower drug level (10^{-6} M as compared to 10^{-3} M), as to preclude the possibility that a decrease in phosphorylation levels is a factor in vivo. Adenine arabinoside and quinine inhibited both phosphorylation and polymerization, with quinine affecting the chloroquine-resistant strains more than the chloroquine-sensitive strain, for both phosphorylation and polymerization. Adenine arabinoside was preferentially more effective against polymerization in the sensitive strain. However chloroquine and primaquine were found to be ineffective against either polymerization or phosphorylation, except at very high doses. The action of chloroquine has long been suspected to be somehow associated with the pigment accumulation that occurs in treated, susceptible strains. War-

hurst et al (1972) found that this accumulation of pigment is due to a buildup of haemozoin. Chloroquine inhibits acid secretion into the digestive vacuole, thus preventing digestion of hemoglobin. This clumping can be inhibited and even reversed by the addition of quinine and several other anti-malarials to the system. This is due to the drug receptor (a membrane bound protein) interacting with compounds that have in common a planar ring of 30-40 Å² with a protonated group either in the ring or nearby. Therefore, compounds with a similar structure to chloroquine can compete for the receptor site and prevent chloroquine binding (Fitch, 1972). In addition to causing reduced hemoglobin digestion, chloroquine, along with quinine and primaquine, has been found to limit the uptake of certain amino acids (Sherman and Tanigoshi, 1972), probably through some inhibition of energetics rather than actual blockage of uptake. Chloroquine and quinine have also been shown to inhibit the enzymes phosphoenolpyruvic carboxylase and carboxykinase, and thus affect energy use (Siu, 1967). Chloroquine has also been reported several times to bind to nucleic acids which would tend to inhibit proper replication (Parker and Irwin, 1952; Allison et al, 1965). Another common anti-malarial drug, pyrimethamine, has been shown to be a potent inhibitor of dihydrofolate reductase, an enzyme that is necessary for production of various folic acid derivatives used in nucleic acid and protein synthesis (Gutteridge and Trigg, 1972). Since then, it has been shown (Kinnamon et al, 1976) that anti-folate drugs can act synergistically, resulting in better control than the sum of the two drugs separately. Using sulfadiazine and a variety of other anti-folates, they showed that there are three possible interference points in the production of tetrahydrofolate dependent products from p-amino-benzoic acid (PABA), and that by attacking two sites simultaneously a synergistic effort could be achieved. Synergistic effects of combined drug therapy have been recorded elsewhere (Schmidt,

1973 and Thompson and Werbel, 1972), and as Kinnamon notes, "Superior activity against drug sensitive parasites, broader action against drug resistant strains, less likelihood of permitting the acquisition of resistance than when the components are used alone, and lower host toxicity (since usually lower doses are required), are benefits noted."

As seen above, using combined drug therapy can help reduce the problem of resistance. Drug resistance, as defined by the W.H.O. is "the ability of a parasite strain to multiply or to survive in the presence of a drug at concentrations that normally kill parasites of the same species or prevent their multiplication. Such resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding maximum doses tolerated by the host)", (W.H.O., 1963).

IV. Problems of Resistance

During WWII, malariologists discovered they had an effective tool in the fight against Anopheles. The early enthusiasm waned, however; with the discovery in 1951 of a strain of flies that were resistant to the effects of DDT. Next came reports from Greece and Latin America that strains of Anopheles were developing there that were also unaffected by DDT. Officials of the W.H.O. received appeals from the Pan American Sanitary Conference in 1954 for a continental malaria eradication program for the entire Western Hemisphere. Malariologists argued for a full-scale attack on the problem, reasoning that if a few lesser or piecemeal programs were undertaken the effect would be to breed a greater number of resistant strains of mosquitoes. They have been hampered in these efforts by a number of different types of recalcitrance to spraying. In some areas of the developing world, governments may not be easily induced to participate, or their mechanisms for carrying out the programs are so inadequate that they cannot accomplish the goal. Also, conservationists tend to oppose the use of residual pesticides,

and DDT in particular, on the grounds that they degrade the environment. Although a case may be made against such compounds, so far as malaria eradication programs are concerned they represent the most hopeful means of attacking a major threat to the health and well-being of people the world over. The World Health Organization has argued strongly against bans on DDT on the grounds that it would expose many people to new malaria risks. In any case, the political resistance and the more technical problem of resistant strains of Anopheles argues for continued research into new compounds. It is possible that a technological solution can be found for the political problems in new insecticides that meet both environmental and vector control needs. However, given the rapid increase in the number of resistant species that appeared just in the period from 1958 to 1970 (from 35 to 104, of which at least 18 were resistant to organophosphates Brown, 1971), it is possible that even if given a free rein, resistance could outmode any new insecticides even before testing is complete. According to the Weekly Epidemiological Record, a publication of W.H.O., of January, 1977 (for data up to Dec. 1975), resistance to DDT had developed in 44 areas worldwide; to Dieldrin - 60 areas worldwide; to Malathion and Propoxur - 4 areas in Central America and one in India; to hexachloro-cyclohexane and Fenthion - one area in India.

In the W.H.O. publication, Manual on Practical Entomology in Malaria, (1975), they discuss the probable genetic reason for DDT resistance and an outline of what to do should resistance develop. Mosquitoes have a genome consisting of two pairs of autosomes and one pair of sex chromosomes. Resistance to DDT, appears to be due to an inversion on the right arm of autosome number 2, resulting in an increased ability to metabolize DDT and an increased lipid concentration, which serves as a storage vehicle for the DDT. They also point out that the extreme irritability that DDT has for sensitive strains can act as a behavioural type of resis-

tance, by causing them to leave or avoid a sprayed area before they receive a lethal dose. If resistance does develop, it should be remembered that larval stages do not always show the same patterns as adults, so that what might be ineffective for one, could still be effective for the other. Other factors to consider before actual spraying is begun are variations in temperature and humidity, (i.e. DDT is less effective at high temperatures, whereas the opposite is true of O-P's), and the timing of application as well (mosquitoes are more resistant to DDT in the morning than they are in the evening). If resistance does develop (i.e. more than 20% survival on 4% DDT for one hour), the following steps should be taken: 1) maintain total coverage, 2) select average indicator villages, 3) install window traps, 4) search for endophillic mosquitoes in DDT sprayed houses, 5) classify captured females according to stomach and ovary status, 6) carry out susceptibility tests at start and finish of spraying program, and 7) correlate known malaria attacks with spraying schedule.

The problems of resistance have also been encountered with the various Plasmodial spp. as well, but particularly with P. falciparum. According to the Weekly Epid. Rec. cited above, resistance to chloroquine in P. falciparum has been found in 4 areas of S. America and in 9 areas in India and Eastern Asia. Resistance has also been found for pyrimethamine and/or proguanil in certain localities of all endemic areas, including Africa. This widespread pyrimethamine resistance is felt to be the result of two factors - massive use of cooking salt with the drug in it, and its use for mass prophylaxis. Both of these helped expose malarial populations to sub-optimal doses, which caused selection pressure to remove susceptible individuals, resulting in a resistant population.

Although the mode of action for resistance in Plasmodium spp. is not well defined for all drugs, we can get some insight into two of the more

widely used drugs, chloroquine and pyrimethamine. Pyrimethamine, as noted above, is a dihydrofolate reductase inhibitor. Jacobs (1964) found that a pyrimethamine resistant strain of P. berghei required a higher concentration of PABA, the folic acid precursor. It would therefore seem plausible that this strain overcomes pyrimethamine blockage by "overloading" the system with excess starting material and is thus able to compensate for slowed folic acid production. If this model is correct, it adds more justification for the use of synergistic drugs. In the case of chloroquine resistance strains, Peters (1970) showed that the uptake of chloroquine by RBC's parasitized by a resistant strain is greatly reduced. As shown above, chloroquine uptake is due to a high affinity receptor site, so a possible model for chloroquine resistance would be an alteration of this receptor protein.

It is readily evident, then, that the classical methods of malaria control are replete with difficulties. Besides the high cost of developing and deploying new anti-vector and anti-malarial measures, once done, there is no guarantee that resistance will not appear nullifying the whole effort. It would, therefore, be greatly advantageous to develop an effective, safe vaccine that would give the host life-long protection. It is not surprising that much of the research effort being expended is in this very promising direction.

V. Human Factors and Malaria

Whenever a disease like malaria is considered, one must not lose sight of the human elements of the disease, as these can have considerable affect on the outcome of any eradication program. A. F. Wesson (1972) listed several factors that need to be considered before any type of program is instituted; income level, education level, degree of social deprivation, migration patterns and local mobility, differential

exposure to vector species, alterations in the physical environment associated with development programs, and cultural attitudes towards mosquitoes and malaria. Of these factors, migration and mobility patterns and alterations in the environment due to development are probably of the most importance.

The W.H.O. bulletin (1974) on Personal and Community Protection discusses the two main categories of migration. First there is migration of laborers toward planned construction sites (these include dams and lakes for hydroelectric power and/or irrigation projects, roads, and railways, etc.). Since such projects require a large labor force, little of which can be filled through locals, most must be imported. The second category involves people who are moving into areas that have recently become habitable (due to clearing of jungle, construction of roads or irrigation projects, or possibly those who have been displaced by construction of dams and lakes). While both involve populations of people who are possibly susceptible to diseases of the new area, or are themselves carriers of some potential pathogen, the individuals in the first category are in a much better position. This is due to the fact that as laborers move into an area, the construction managers organize the people into a hierarchial community, with provisions for health care and sanitation procedures. This is not a completely altruistic process, as the list of failures of construction projects due to epidemics of various diseases raging through a susceptible labor force is quite long. As the bulletin states it, "it is essential, therefore, that the capital investment for the project should include funds for safeguarding the health of the community in the project area, particularly against malaria." The second group, however, are often left to fend for themselves, and often in an area where the available health services are non-existent. The bulletin suggests, therefore,

that surveys be completed before such areas are opened up for settlement to determine the degree of malaria risk. Once established, each housing unit should be sprayed yearly with a residual spray and the proper drugs should be disseminated (one idea - in an area where the population is rather elusive - is the distribution of drugs through the use of medicated salts). These methods should be used in conjunction with the use of mobile health teams, or if these are non-existent, some type of collaboration with the army should be used.

In addition to the various movements of people that can be caused by various construction projects, it must be remembered that these projects are also responsible for altering the ecology of an area. The building of a road or railroad, if proper drainage work does not accompany such construction, can greatly increase the breeding habitats of many potential vectors. Even the clearing of a forest or jungle can cause an increase in malaria risk. As E. E. Gordon Smith (1972) points out, forest clearance can force a previously zoographic species (Anopheles fluviatus) into adopting an anthropophagic pattern, or it can allow an increase in the numbers of sunlight breeders (A. quadrimaculatus - a very important vector). Probably the most potential harm can come through the building of large water projects, dams and irrigation systems. One of the more complex problems of management has been caused by the building of the Aswan dam. The accompanying lake (Lake Nassar) has allowed a direct extension of the breeding grounds for A. gambiae from Northern Sudan into Southern Egypt. Previous spreading of this vector on various occasions has always resulted in devastation of the largely unprotected population. Control has always been accomplished in the past by vector eradication, however, this may no longer be possible. It is therefore of the utmost importance to be aware of all possible consequences when major construction projects are

planned. As H. E. Patterson (1972) points out, when building dams (and these could be applied to other projects as well), there are two key questions that should be asked, 1) how is the particular dam under construction likely to influence mosquito production, and 2) what are the health or nuisance consequences of the above. One aspect of mosquito control can be aimed at larval control. According to the Manual on Larval Control Operations in Malaria Programmes (W.H.O., 1973) the first step of any program is information studies on 1) environmental conditions, 2) anopheline fauna with special reference to vectors and suspected vectors in the area, 3) ecology of vectors and 4) season of transmission. Only after informational studies are completed should adult and larval studies be done on 1) anopheline fauna with special reference to vectors and suspected vectors, 2) locate and identify preferential breeding sites and classification of same and 3) collect information on suitability of some larval sites and adult day-time resting shelters as future fixed capture stations. Such studies would be of extreme importance in predicting fauna changes due to a major environmental modification such as a dam, and would thus be invaluable in any type of a control program.

The importance of proper identification is noted by Patterson when he cited the example of A. gambiae, an extremely important malaria vector, which for many years was considered to be a single species, but which is now recognized to have at least six varieties. The importance of positive identification becomes apparent when it is realized that three of the varieties are freshwater breeders, while three breed in brackish water, thus any control program would have to be cognizant of the differential breeding habitats of these varieties. Another example is found in Malaya, where along the coasts the principle vector is A. umbrosus. This species breeds in almost stagnant water, that is

heavily shaded by mangrove, so that a program of clearing and drainage would be the best means of control. Such a clearing and drainage scheme would be anti-productive on the interior of the country where the primary vector is a clear water, bright sunshine breeder, A. maculatus.

In addition to management problems caused by new bodies of water, many low-lying fertile areas are made uninhabitable by the presence of a high malaria risk. The usual response to control of such areas is a process of clearing and drainage. But as G. T. Carmichael (1972) points out, water management does not always mean drainage. He lists five possible means of control: 1) introducing salt water to control Anopheline ovipositing and larvae, 2) fluctuating water levels to prevent still water pools, 3) keeping water high enough to maintain larvivorous fishes, (4 control of sanitation water, and 5) quick draining of any type of flood water. The coupling of the fifth method with a larviciding program was successful in reducing the number of cases in Haiti (1969-70) from 1500 to 9. Another method that could be of importance to coastal regions was one used successfully in El Salvador, where the tides (and salt water) were allowed to penetrate the interior waterways and thus control the primary vector, A. albimonus which is an open sunlight breeder but with a low salt tolerance.

There is another aspect in today's world that is gaining more and more importance in the transmission of communicable diseases. With international travel becoming more accessible to more and more people, the chances of unprotected travelers coming into contact with a variety of diseases is becoming much greater. However, there is much greater danger to be found in the possible spread of vector species into previously uninhabited regions, such is the case of Guam. Prior to WWII there were no indigenous Anopheline species found on the island, However, post WWII and up until 1970, A. indefinitus was the only species

present. Since 1970, 8 species have been introduced, and one, A. litoralis, has been indicated as a vector of both falciparum and vivax malaria, (Ward et al 1976). It is therefore of extreme importance that a close watch be kept on possible importation of possible vector species and control measures taken where needed.

VI. Immunological Aspects of Malaria

As early as 1924, it was recognized that "the failure of treatment in chronic relapse cases is explicable on the same hypothesis (insufficiency of immune body formation); the parasites in such cases are not quinine-resistant, but immune-body resistant," (Yorke and Macfie, 1924). As previously noted, one of the clinical signs of malaria is an enlarged, palpable spleen. This hyperplasia is due to the response of the lymphoid tissues of this organ - both lymphocytes and reticulo-endothelial tissues of this organ - both lymphocytes and reticulo-endothelial macrophages (Taliaferro, 1967). To determine whether or not immunity to P. berghei could be transferred to an unprotected individual, Phillips (1970) prepared lymphoid cells of diverse origins, (spleen cells, peritoneal exudate cells, lymph nodes, thymus, and bone marrow) and plasma from immune mice. Although some protection was developed through the transfer of serum plasma alone, cell-transfer was much more effective in producing an immune state. The importance of the thymus in this response is shown in a report by Stechschulte (1969), who, after performing a thymectomy in neonatal rats, showed that the rats had a decreased ability to respond to a challenge of P. berghei. G. L. Spittlany (Spittlany et al, 1977) took this process one step further. Using nude mice (congenitally lacking a thymus), he found that a challenge with P. berghei sporozoites was always lethal. However, if before the challenge, he transferred T-cells from immuno-competent individuals, the challenge was not lethal. He attributed this protection

to an increased macrophage migration ability.

A second immune response to most diseases is production of antibodies (Ab's) by B-cell lymphocytes. Malaria is no exception, as Ab production is not suppressed in most infections, however, the effectiveness of these Ab's seems to be minimal to moderate at best. Smalley and Sinden (1977) found that in a P. falciparum infection, the level of circulating Ab had no correlation with the level of circulating gametocytes. A patient with the highest Ab titer, was found to still be infective to feeding Anopheline mosquitoes. They also showed that when the level of gametocytes does begin to fall, this decline is independent of the quantitative or qualitative levels of anti-gametocyte Ab's. The idea of qualitative differences in Ab populations is supported by McGregor and Carrington (1961), who showed that Ab's against P. falciparum trophozoites were ineffective against the gametocytes. It appears that the removal of gametocytes is accomplished primarily by the spleen in its function of removing and destroying old, damaged, and malformed RBC's (Wintrobe, 1974), however, Ab production is not without some value. Hamburger and Kreier (1976) showed that Ab's only attach to eroerythrocytic merozoites and not to erythrocytic merozoites. Although this attachment does not appear to cause any actual damage to the merozoites, Ab attachment does appear to inhibit the merozoites ability to bind to new RBC's (thus preventing further invasion), and Ab attachment does seem to increase opsinization (attraction and ingestion by macrophages).

As noted above, our association with malaria probably extends back many, many years. Not only have humans developed selective traits against them, but they have developed protective traits against us as well. The malarial response to an immune reaction covers a wide range, from immunosuppression to hyperimmunoglobulinemia. In 1975, Greenwood

and Vick found that P. falciparum (or the infected cells) release a mitogenic-like factor, thus causing an increased but non-specific Ab production (hyperimmunoglobulinemia). This was extended by Tanabe et al (1977) who found that P. berghei caused a suppressed response of specific Ab cells in mice. They proposed the following mechanism: first, there is a depletion of T-cells; second there is a non-specific mitogenic stimulation of B-cells; then these two effects combine to produce the third effect, which is to disrupt specific interactions with antigen handling macrophages, thereby preventing a specific response.

In addition to this non-specific stimulation, Poels et al (1977) found that P. berghei release so-called "protective" antigens that are released by the growing stages within polychromatic erythrocytes. These would act more or less like "decoys", and thus, once again, prevent a specific Ab response, but there is also a type of immunosuppression as well. Poels and Niekirk (1977) point out that malarial infections are known to depress immune responses to a diverse group of antigens (sheep RBC's, skin grafts, lymphomagenic viruses, and various other infections - probably all related to the T-cell depletion mentioned above). By measuring IgA production in P. berghei infected mice, both before and after chloroquine treatment, they found the level of IgA rose after treatment.

Although there are real problems then with depending on our immune systems, there are advantages as well. If a successful vaccine could be developed, the problems of resistance, and the great expenses of spraying and treatment could be forgotten. As yet, vaccine development has been limited primarily to animal model systems. Holbrook et al (1976) found that they could stimulate a small response to P. berghei sporozoites in mice by prior injection of formalin killed merozoites. By far

a greater response has been produced by Richards et al (1976), who, using a P. knowlesi - rhesus monkey system, were able to produce definite protection against a sporozoite challenge by inoculation with a merozoite vaccine. However, this vaccine requires the use of Freund's complete adjuvant and would therefore be of no use in man. Along this same line, Clark, Cox, and Allison (1977), found that intravenous intraperitoneal injections of killed Corynebacterium parvum into mice conferred protection against a normally lethal challenge of P. berghei. However, this was not an Ab response, but due rather to some enhanced response of some cellular mediator.

Advancement towards development of a human vaccine has also been made in the last two years. With the successful transfer of P. falciparum to the owl monkey, researchers no longer have to rely on human volunteers, but this still leaves the problem of gathering enough material to do vaccine research or production. This was solved by the development by W. Trager (1976) of the first continuous culture of P. falciparum; the media uses human RBC's, RPMI Medium 1640, 7% CO₂, 1-5% O₂, and a 38 degree C. temperature. Although they lose all synchrony, they remain infective. Finally, there is the discovery of Richard Carter and David Chen, (1976). Working with chickens infected with P. gallinaceum, they collected blood and, by a series of centrifugations were able to collect a fairly pure suspension of gametes (not gametocytes). These were then irradiated (20,000 rads) and injected into previously unexposed chickens (3 injections of 1.5 ml./injection/week). After about one month, they exposed the chickens (with anti-gamete Abs) to mosquitoes. Whereas the feeding of 10 mosquitoes per day through the infection would normally produce from 2,000 to 4,000 oocysts, these produced 1 or less. This was all accomplished without the use of adjuvants - an absolute pre-requisite for

any human vaccine, and as they point out, this discovery can have extremely important aspects for other vector borne diseases as well.

Summary

Although the etiology of this disease has been known for almost 100 years, and fairly effective means of control have been available for over 30 years, malaria still affects millions of people each year. This survivability is due to a number of factors, including development of resistance of the vectors to previously effective insecticides, resistance of the Plasmodium spp to anti-malarials, and possibly of greatest importance, is the ability of the vector populations to take advantage of the environmental changes made by man. Proper planning and common sense can be of extreme importance in the control of this disease. New approaches, including use of biological control agents and the possible development of effective vaccines are control measures that will assume greater and greater importance in the future.

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CONTROL AND ERADICATION OF
PLASMODIUM SPP. IN MAN

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B.S., Kansas State Univ., 1973

AN ABSTRACT OF A MASTER'S REPORT

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Parasitology

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1978

ABSTRACT

Although malaria has been associated with man for many millenia, it has only been within the last 100 years that the etiology of the disease has been known and only within the last 30 years that practical methods of control have been developed.

The disease is caused by organisms of the Phylum Protozoa that have been placed in the genus *Plasmodium*. There are four species found in man: *P. ovale*, *P. vivax* (tertian malaria), *P. malariae* (quarter malaria) and *P. falciparum* (malignant malaria). Of these four, the first three cause characteristic periodic symptoms of fever and chills. This is due to the synchronized release of the stage that infects red blood cells (RBC's), the merozoites. The fourth type, *P. falciparum*, is generally asynchronous and as a result, is generally more dangerous. Without the associated fever and chills, it is possible to be remiss in seeking treatment. In addition, it is common for RBC's infected with this type of malaria to tend to stick together and cause blockage of capillaries in vital organs (brain, kidney, and lungs).

The disease is dispersed from man to man by the use of a biological vector. The arthropod vector, mosquitoes of the genus *Anopheles*, is essential to completion of the parasite's life cycle. Not only do developmental changes occur, but multiplication of numbers occurs as well.

Because the disease is dependent on existence in two hosts, this allows a two-pronged attack on the disease. However, such an attack has only become possible since the end of WW II, with the deployment of the first effective residual insecticide, DDT. Also, development of effective drugs for treatment in man (without the serious side-effects caused by quinine) have aided in control programs. It is now becoming possible to investigate the modes of action of many of these anti-

malarials, which should further aid the development of even more effective drugs.

One of the drawbacks in the widespread use of both residual insecticides (DDT, malathion, Dieldrin, etc.) and anti-malarials is the problem of resistance. Resistance is the ability of a small percentage of a formally sensitive population to survive a normally lethal dose of some drug. This small percentage then becomes the dominant phenotype, with the result of negating the control program. There is also the problem of environmental degradation with the use of residual pesticides. These factors must be taken into consideration when developing any type of control program.

Other factors that must be considered include the human factors of the disease. Increasing population mobility (with the possible movement of susceptible or diseased populations), increasing urbanization and modernization (with resultant changes in the environment causing vector changes), and increased speed of transportation can all have an affect not only on malaria but other communicable diseases as well.

Because of the many problems encountered with the conventional methods of control, there is increasing research into the possibility of developing an effective vaccine. This would be an extremely valuable tool, but due to the many forms that are found in man (merozoites, sporozoites, gametocytes) such an approach has so far been found impractical. However, recent development of a vaccine that might be used to combat the protozoan while in its arthropod vector offers exciting and promising advances in the control of not only malaria but all vector borne diseases.

Although the dream of eradication has not yet been achieved, many advances have been made in the control of this ancient disease. With

intelligent, balanced attacks on both the disease and its vector, and through the proper use of chemicals, improved sanitation, manipulation of vector modernization, this scourge will be added to the growing list of forgotten diseases.