INDIAN JOURNAL OF MALARIOLOGY

Volume 21 Number 1

June 1984

MALARIA RESEARCH CENTRE
Indian Council of Medical Research
22, Sham Nath Marg
Delhi-110 054

INDIAN JOURNAL OF MALARIOLOGY

HALF YEARLY

SUBSCRIPTION RATE

Annual subscription	India	Rs. 30.00*
	Other countries (including airmail postage)	$ 10.00

*50% Discount would be admissible to the individual subscriber on annual basis.

Payments in respect of subscription may be sent by money order/bank draft/postal order crossed and marked payable to Malaria Research Centre, 22, Sham Nath Marg, Delhi- 110 054.

INDIAN JOURNAL OF MALARIOLOGY

INDIAN JOURNAL OF MALARIOLOGY

CONTENTS

Volume 21
Number 1
June 1984

Short Note

Book Review

Note: The editor assumes no responsibility for the statements and opinions expressed by the contributors. This issue has been delayed for reasons beyond our control.

Indian Journal of Malariology
Vol. 21, June 1984, pp. 7-16.

Chemotherapy of Malaria and the Host-Parasite Interaction

WALLACE PETERS[1]

INTRODUCTION

Browsing through my library shelves recently, my hand fell on a dog-eared journal just 25 years old. It was the Silver Jubilee number of the Indian Journal of Malariology, and I recalled that it was given to me by the great malariologist, the late Colonel Jaswant Singh shortly after my wife and I returned home after helping to initiate the antimalarial programme in the Nepalese Terai. The articles in that journal make fascinating reading today, and provided me with an excellent stimulus for reflecting upon problems resolved and unresolved since 1955, and the new problems that have arisen since then.

The first article was by Sinton (1955), who drew attention to some of the gaps in our knowledge of the malaria parasite. These are some of the questions he posed. Is the malaria parasite inside or outside the erythrocyte? (This was the year that Fulton and Flewitt demonstrated for the first time, by electron microscopy, that the trophozoites are intraerythrocytic). Have we a proper picture of the cytological structure of the malaria parasite? What is the nature of the changes in the erythrocytes infested by malaria parasites? What is the fate of the sporozoites in the insect host? (He might well have added—"and the vertebrate host"). Is there a malaria toxin? What is the nature of "antibodies" in malaria? What is the origin of gametocytes? The advent of electron microscopy and quantum advances in immunology have produced partial, but not total answers to these questions, and recent studies guided by Garnham (Krotoski *et al.*, 1980) have finally thrown light on the origin of relapses in vivax malaria. None of this, however, has yet enabled us to overcome the scourge of malaria, as India knows only too well.

That 1955 Jubilee number with articles also by Jaswant Singh, George Macdonald, Pampana, Garnham, Christophers, Covell, B. A. Rao, Knipe, V.V. Rao, D.K. Viswanathan, Rajinder Pal, M.I.D. Sharma and others, was indeed a vintage publication. It appeared at a moment when the great Indian DDT campaign was well under way, when great expectations were held that the land would soon be freed from the burden of malaria, and when "eradication" was a respectable and indeed revered word. Alas, the dream was unfulfilled. From a great start that culminated in a dramatic fall in transmission by 1960, the campaign fizzled out, and the net result was that epidemic malaria, fulfilling all the direst theoretical forecasts of Macdonald (1957), returned at an exponential rate so that, by 1976, at least 6.4 million cases had been recorded by

Accepted for publication : 9 November 1983.
[1]Department of Medical Protozoology
London School of Hygiene and Tropical Medicine
Keppel Street, London WC1E 7HT, England.

the National Malaria Eradication Programme (NMEP), (Ray, 1977) and an emergency control programme had to be inaugurated (Ray, 1979). In 1980, there were still at least 2.2 million cases and the proportion of *P. falciparum* was increasing. Jaswant Singh (1955) cautioned that, at that time, "...it would appear that the main problem in the treatment of malaria has practically been solved. But on deeper consideration, it becomes evident that there are still many lacunae in our knowledge of the subject." Later he said "In order to achieve this objective *(of finding new drugs)*, it would be essential to understand clearly the metabolic processes of plasmodia at all the phases, particularly the tissue and blood forms."

Since 1955, we have learned a great deal about the structure and cell biology of malaria parasites (see reviews by Sinden, 1978; Aikawa and Seed, 1980), their biochemical processes (Sherman, 1979) and the nature of the host's immune response to malaria infection (Nussenzweig *et al.*, 1978; Cohen, 1979; Kreier and Green, 1980). New antimalarial drugs have been developed to combat the rapidly increasing problem of multiple drug resistance in *P. falciparum* (Peters, 1970, 1974, 1980a; Canfield and Rozman, 1974; Rozman and Canfield, 1979), and remarkable progress has been made towards the production of antimalarial vaccines (McGregor, 1979). Nevertheless, we are still far from winning the struggle against *Plasmodium*, and have much to learn from an analysis of past experience in order to make the best use of continuously shrinking biomedical research resources in the future. Some of our current knowledge is discussed in the present paper in the hope that its consideration will throw up ideas for future investigations on antimalarial drug development.

VITAMINS, HOSTS AND PARASITES

Sir Edward Mellanby, in whose memory this paper is presented, together with his mentor Hopkins at Cambridge helped to pioneer the new field of the vitamins at the beginning of this century, and made important contributions especially to our knowledge of the mode of action of vitamins A and D on mammalian tissues. Within a few years, early knowledge of the importance of vitamins for cellular integrity was also being applied in the development of culture media for malaria parasites (Bass and Johns, 1912). Interest in this topic waxed and waned, with periods of peak activity during World War II and during the 1970's when, at last, Trager and Jensen (1976) succeeded in obtaining continuous cultures of the blood stages of *P. falciparum*. Huff *et al.* (1960) had in the meantime grown the exoerythrocytic stages of *P. foliax* in tissue culture. From these and the many intervening attempts to culture different stages and different species of malaria parasites *in vitro*, a wealth of information had accumulated about the basic vitamin requirements both of the parasites and of their host cells (Von Brand, 1973). As a side note, it may be mentioned that neither of the vitamins that most interested Mellanby, A and D, are essential to *Plasmodium* although they are, of course, to their hosts.

The vitamins that are of particular importance for the metabolism of malaria parasites and for a number of kinetoplastid flagellates are *p*-aminobenzoic acid, biotin, pantothenate, folic acid, nicotinamide, pyridoxine, riboflavin and thiamine (Trager, 1977; Taylor and Baker, 1978). Although little attention appears to have been paid to vitamin K, work that will be discussed in this paper suggests that this compound too should be of considerable importance to parasite respiration. The knowledge of the significance of these essential growth substances to *Plasmodium* gleaned from cultivation studies has led attempts to design antimalarial substances that can block their uptake or utilisation by the parasites. Unfortunately, most such compounds have proved equally effective in blocking the use of these essential vitamins also by the hosts. Other, retrospective work has

attempted to explain the mode of action of various antimalarials, e.g. mepacrine, in terms of their blockage of vitamin uptake by *Plasmodium*, in this particular example, riboflavin which Rao and Sirsi (1956) showed was essential to the growth of *P. gallinaceum*.

This, in fact, is probably not the way this drug works (Peters, 1970). This lack of specificity has led to a search for differences between the metabolic pathways of malaria parasites and their hosts, with the objective of exploiting such points with what then might prove to be selectively toxic agents. The results of this approach will be referred to later in this paper.

It is essential at this point to underline one factor, fundamental to all chemotherapy, namely that there is perhaps no known chemical agent that will completely free the host from an invading organism without the active participation of the immune defence mechanisms of that host (Peters, 1980b). In discussing the chemotherapy of malaria, therefore, it is necessary to consider both the nature of host immunity against *Plasmodium*, and the possible influence of various drugs on those immune mechanisms, as well as on the parasites themselves to understand how the host immune responses are triggered, it is essential first to dwell briefly on the nature of the host-parasite interface.

THE HOST-PARASITE INTERFACE IN MALARIA

During their life-cycle most malaria parasites pass from a poikilothermic insect host, the female *Anopheles* mosquito, to a warm blooded vertebrate host (parasites of reptiles are an exception), an ecological change that necessitates major shifts in their metabolic pathways (Howells *et al.*, 1972). Moreover, the parasites in each type of host pass through relatively immobile growth phases, sporogony in the mosquito, exo- and intraerythrocytic schizogony in the vertebrate host, with motile phases connecting one schizogonic stage with the next. All the motile stages, with the exception of the microgamete, are characterised by possessing a protective surface layer, and a subpellicular skeletal structure. Their energy requirements are probably provided by stored substances, e.g. in the spherical body of the erythrocytic merozoites and the crystalloid body of the ookinete, and any further nutritional requirements are likely to be acquired by simple diffusion. The motile stages may thus be particularly insusceptible to chemical attack until the moment arrives when they invade a new host cell. They are, however, vulnerable to immune attack by antibodies (Cohen and Butcher, 1971) and perhaps also by activated macrophages or other white cells.

The immobile schizonts of the mosquito (the oocysts on the outside of the midgut), and of mammalian parasites (primary and secondary exoerythrocytic schizonts growing in hepatocytes) share many structural features, both appearing to absorb nutrients from their environment. They are apparently protected against most chemical agents with a few notable exceptions, namely sulphonamides and dihydrofolate reductase inhibitors (antifols) that block pathways leading to purine and pyrimidine biosynthesis, 8-aminoquinolines, and certain naphthoquinones (e.g. menoctone) that may, either directly or through metabolites, interfere with parasite respiration

These compounds also inhibit the growth *(a)* of the exoerythrocytic schizonts of avian *Plasmodium* such as *P. gallinaceum* in cells of the reticuloendothelial system and *(b)* intraerythrocytic asexual stages of all species. In addition the latter are acted upon by certain compounds such as chloroquine that, for some still incompletely defined reason, are strongly and selectively bound to them. Recent studies by Chou *et al.* (1980) suggest that chloroquine binds to an intermediary product formed during the degradation of haemoglobin by the parasites to the insoluble malaria pigment, haemozoin.

Probably the least vulnerable of all the stages of the malaria cycle are the hypnozoites of relapsing *vivax*-type parasites of man and other primates (Krotoski *et al.*, 1980). They remain as inactive, unicellular parasites in hepatocytes for months or years before, at some unknown signal, bursting into schizogony to produce a fresh wave of cryptozoites that are destined to invade new erythrocytes, i.e. a relapse. How the hypnozoites in their state of "hibernation" survive metabolically is a mystery at present. Probably, they are able to maintain the minimum level of respiration necessary for survival, either by continuing a low level of anaerobic glycolysis, or by absorbing the essential metabolites from the host cell. All that is known so far is that they are affected by one type of compound only, namely the 8-aminoquinolines, e.g. primaquine. Whether this drug acts directly on the parasite, or on the host cell, remains to be seen.

It was mentioned above that the motile stages may be susceptible to chemical intervention at the moment when they invade a new host cell. The sporozoite, like the hypnozoite, appears only to be affected by 8-aminoquinolines (Terzian *et al.*, 1951) which are, unfortunately, too toxic to be used as causal prophylactics. The fate of the sporozoites is one intriguing aspect of the malaria life-cycle that is currently the focus of attention of several investigators. In rodent malaria, Verhave *et al.* (1980) and Smith and Sinden (1981) have produced evidence strongly suggesting that the sporozoites are actively removed from the peripheral circulation after inoculation by the mosquito, by Kupffer cells of the liver. Recent studies by Pirson *et al.* (1980) suggest that, by "targetting" primaquine into the Kupffer cells through incorporating it in liposomes, the prophylactic activity of the drug is enhanced and the toxicity reduced, (i.e. the selective toxicity is improved, the therapeutic index widened). The biological implications of this work are that *(a)* the sporozoites are first taken up by the Kupffer cells, that *(b)* they subsequently pass from the Kupffer cells to the hepatocytes and that *(c)* primaquine may prevent this passage. However, primaquine is also concentrated in hepatocytes where it may also exert its antiparasitic action.

Hommel (personal communication) has suggested that other antimalarials may act by blocking the entry of merozoites into erythrocytes. It has been suggested that a histidine-rich protein produced in the rhoptries of erythrocytic merozoites (Kilejian and Jensen, 1977) may play an important role in the attachment of merozoites to new red cells or, more likely, in their invagination of those cells (Dvorak *et al.*, 1975). The attachment process which also involves red cell surface receptors and parasite recognition of those receptors (Miller *et al.*, 1975), and subsequent invagination are blocked *in vitro* by concentrations of chloroquine in the order of that found in the serum of patients (Hommel, *loc. cit.*). Another possible mechanism by which such blood schizontocides may act is by concentration within the rhoptries (Warhurst and Thomas, 1975) and subsequent interference with the function of the rhoptry contents.

BIOCHEMICAL PATHWAYS AS DRUG TARGETS

Like many bacteria and unlike vertebrate tissues, malaria parasites are unable to incorporate preformed folate and must synthesise it *de novo* from *p*-aminobenzoic acid (PABA). This situation offers a classical target for selective drug toxicity and explains the potent action of many sulphonamides and sulphones on the actively metabolising stages of *Plasmodium*. It is fortunate that the enzymes concerned with the later stages of folate metabolism, e.g. dihydrofolate reductase (DHFR) differ in many physico-chemical characters from those that perform essentially the same functions in the vertebrate hosts. Thus the affinity of pyrimethamine for the DHFR of *P. berghei* is some 10^4 times greater than for that of the mouse (Ferone *et al.*, 1969). Other enzymes in the folate

pathway have barely been exploited and almost certainly offer other opportunities for the development of selectively toxic inhibitors (Ferone, 1977). A logical sequel to having two compounds that affect sequential steps on a single pathway is to apply them together. Thus a combination of sulphadoxine which blocks the utilisation of PABA, and pyrimethamine which blocks DHFR provides a marked degree of drug potentiation which has proven of considerable practical value in malaria chemotherapy (Peters, 1970). The potentiation is of such a level that it is able to overcome the loss of activity by each component when used individually against certain drug-resistant parasites (Peters, 1968), and to delay greatly the development of resistance to the individual drugs by originally sensitive parasites (Peters, 1974a). Unfortunately, this is not entirely a preventive effect but only a delay, and an increasingly large number of strains of *P. falciparum* are appearing in the field that are resistant to a currently used mixture of this type (sulphadoxine-pyrimethamine, Fansidar) (CDC, 1980).

Up to a point, apparent resistance to these mixtures may be due not to true drug resistance, but to a pharmacogenetic peculiarity of the host. Normally the effective "half-life" of each component of these mixtures is similar. Certain individuals, however, metabolise and excrete long-acting sulphonamides more rapidly than others (so-called "fast-acetylator" phenotypes) (Gilles and Clyde, 1974). Thus these individuals receive protection only from the antifol component for part of the anticipated period, and this may be insufficient where antifol-resistant parasites are present. Bjorkman *et al.* (1980) have suggested, that idiosyncratic host factors in relation to the antifol components may also give a false impression of drug resistance.

The important role of pantothenate in the metabolism of *Plasmodium* has been well documented by Trager (1977). The work of his collaborators and himself highlighted an important aspect of the intimate host-parasite relationship between the erythrocytic stages and the red cells, namely the dependence of the parasites on the host for certain biosynthetic processes. *P. lophurae,* for example, was found to be deficient in coenzyme A (CoA) for which pantothenate is a precursor (Bennett and Trager, 1967). Thus pantothenate deficiency, or pantothenate antagonists were shown to have a marked antimalarial effect against several species of malaria parasites *in vitro*. As Trager (1977) pointed out, the detection of other "biosynthetic lesions" could offer new, selective targets for drug action. The problem here, of course, is that drugs affecting host enzymes on which the parasites depend may also adversely affect the hosts themselves. Nevertheless, there may be certain situations in which a marginal decrease in the level of host enzyme (e.g. pyridoxine kinase) may just suffice to damage the parasites without influencing the host. Genetic differences in the level of this enzyme in the blood of Africans and whites, for example, may contribute indirectly to the superior ability of the African to tolerate *P. falciparum* parasitaemia (Chern and Beutler, 1975).

Siddiqui and Trager (1964) drew attention to the manner in which *P. lophurae* may depend also upon folate derived from the host erythrocytes although, in this instance, the parasite does possess its own DHFR. Peters *et al.* (1973) has suggested that increased dependence upon the host cell for certain essential metabolites may be one way in which various types of drug-resistant parasites can survive in the presence of otherwise lethal concentrations of drug. Thus, Howells and Maxwell (1973) showed that chloroquine-resistant *P. berghei* induce their host reticulocytes to produce enhanced quantities of NADP isocitrate dehydrogenase which may permit their survival in the presence of chloroquine. It may be this general ability of intraerythrocytic, drug-resistant *Plasmodium* to induce excessive metabolic activity in their host cells that explains apparent cross-resistance of

such dissimilar compounds as pyrimethamine and chloroquine. Certainly the modes of action of these two compounds against drug-sensitive parasites are quite different (Peters, 1970).

A potentially valuable target for selective drug toxicity in malaria parasites that does not appear to have been adequately followed up is the ubiquinone system that is intimately concerned both in methyl transfer and in mitochondrial respiration. Skelton *et al.* (1969) demonstrated that several *Plasmodium* species contain not ubiquinone-10 as do their hosts, but ubiquinone-9, and it has been suggested that the selective antimalarial action of certain naphthoquinones such as menoctone may be related to this finding (Peters, 1974b). Moreover, there is a body of evidence indicating that 8-aminoquinolines which have been shown to influence mitochondrial integrity (Beaudoin and Aikawa, 1968, Howells *et al.*, 1970) and function (Skelton *et al.*, 1968) may in fact function through metabolic products. Could these include quinolinequinones which also influence ubiquinone-9? Here is an area that seems to be ripe for further exploration both by biochemists and medicinal chemists.

DRUG-IMMUNITY INTERACTIONS

The vertebrate host responds to infection with malaria parasites in several ways, depending upon the intensity and frequency of infection, and the species of parasite. Sporozoite invasion induces a humoral response, anti-sporozoite antibodies being produced that, in time, effectively neutralise homologous reinfection (Nussenzweig *et al.*, 1978; Cochrane *et al.*, 1980). Antibody-inactivated sporozoites are presumably removed from the circulation by Kupffer cells of the liver in much the same way as has been postulated for normal sporozoites. Presumably the difference in the immune host is that the sporozoites are destroyed by these macrophages, whereas they survive to penetrate and grow in hepatocytes in the non-immune host.

Host response to the hepatic schizonts appears to be minimal, although phagocytic invasion can be seen at the site of ruptured, mature schizonts. The major defences seem to be reserved for the intraerythrocytic parasites which produce a mitogenic factor (Greenwood and Vick, 1975), and against these the host launches both a humoral response (IgM followed by IgG), and a cell-mediated response (Cohen, 1979). In this way red cells containing parasites that have been damaged, but not necessarily killed by drugs, are removed from the circulation into such organs as the spleen, in which macrophages remove and finally destroy the parasites, together with the products of their metabolism (e.g. haemozoin which is stored in the macrophages). The presence of a heavy malaria parasitaemia itself has an immunodepressant action which has been amply demonstrated both in experimental models (Salaman *et al.*, 1969) and in man (Greenwood *et al.*, 1972). This is, presumably, a survival mechanism evolved by the parasites, and it is interesting to note that a similar process has been evolved by other blood parasites such as the African trypanosomes (Greenwood *et al.*, 1973). Some antimalarial drugs have not only a direct antiparasitic action, but also an effect on various functions of the host immune system. Thus chloroquine is a well-known suppressor of cell-mediated immunity. The level at which this is effected, however, is far higher than that needed to produce its beneficial antimalarial action. The same is true of primaquine (Thong *et al.*, 1978). While it has been claimed that pyrimethamine is an immunopotentiator (Thong and Ferrant, 1980), it seems unlikely that this plays any role in the antimalarial action of this compound since exceedingly small blood levels are adequate to exert the selective blocking action of pyrimethamine on parasite DHFR.

In recent years attention has been focussed on the non-specific immunity-stimulating activity of several polysaccharides such as those of BCG (Clark *et al.*, 1976), *Corynebacterium parvum* (Clark *et al.*, 1977) and 3 1-3 glucan (Gillet *et al.*,

1980). Moreover, certain glycolipids used for the production of liposomes were also found to exert a prophylactic effect in mice against sporozoite challenge with *P. berghei* (Alving *et al.*, 1979). Such agents produce a non-specific type of protection against a variety of parasitic protozoa, and even some helminths (Cox, 1978). In the light of the intensive efforts currently being made to produce antimalarial vaccines, perhaps these new approaches using drugs as immunostimulants may offer a new road for a combined vaccine drug attack on malaria transmission.

CONCLUSIONS

It would be wrong to think in terms of a single-track approach to the control of malaria. Drugs can contribute enormously to the management of infectious diseases including malaria but cannot eliminate the parasites in the individual, much less in the community. The immunologists like those concerned with chemotherapy think and have tended to see everything through a rose-coloured haze of vaccine. As the burden of malaria transmission becomes less in a community, so does that community require heavier drug dosage for infections that do occur, and so does the level of communal immunity fall (Peters, 1983). Moreover, as immunity decreases, the gametocyte load, and hence, potential infectivity of the community to *Anopheles* mosquitoes increases (Macdonald, 1957). Active anti-vector measures are, therefore, demanded in addition to the application of drugs and possible vaccination of the community. In other words, malaria control must be an integrated operation, tailor-made to the needs as defined by careful malariometric studies of particular ecological situations.

Drug development should equally be tailor-made to fulfil the particular needs of the individual or the community. It is necessary to consider the ecology of the parasites at different stages of their life cycle, to discover their vulnerable points. The response of the host must be taken into account, genetic differences in that response, the possibility of manipulating that response by the design of chemical or biological immunomodulators. Optimal ways of applying new drugs must be sought. What may be adequate to protect an individual may be quite inappropriate for a community. For example, a weekly tablet suitable for a few people may be replaced by an injectable, long-acting repository formulation (Rieckmann, 1967) to protect whole villages. Studies on such formulations are currently in progress under United States Army and WHO auspices.

Few of us have the talents or the good fortune to progress from the detailed, meticulous approach of the laboratory worker, to a position which permits one to take the broad view of research such as that enjoyed later in his life by Sir Edward Mellanby when he became the Secretary of the Medical Research Council. He would have been the first to advocate taking the broad perspective while, at the same time, keeping his eye on the fine details of the research for which he was responsible. We would all do well to bear his example in mind as we seek to overcome the global problem of malaria.

Malaria is a relentless enemy. We see that here in India better than anywhere. Today we have drugs with which we can treat people if they fall ill, but we *do not have a single preparation upon which we can rely to protect the vast communities* to whom malaria is a threat, and this is particularly true where drug resistant malignant tertian malaria is spreading.

Thanks to a unique and costly effort of our colleagues in the United States, particularly those in the Walter Reed Army Institute of Research; thanks to the current programme of the WHO/UNDP/World Bank Special Programme for Training and Disease Research; thanks to the persistent effort of two or three pharmaceutical research organizations in my own and other countries; and last, but by no

means least, thanks to the work of scientists in your own CDRI, we are developing several potent new drugs with which to combat malaria, and the appropriate formulations in which to administer them to the masses, not just to individuals. We must deploy the new weapons to the best advantage, together with new insecticides where the old ones have failed, together with education of the people in simple measures for their own protection.

It will be a long hard struggle to control malaria in India, but let us try once more; let us learn from past mistakes and not expect miracles through regarding a complex problem as one for which there is any simple solution.

ACKNOWLEDGEMENTS

I wish to express my great appreciation to Dr. Nitya Anand and the Administration of the Central Drug Research Institute, Lucknow, for the honour of my invitation to present this, the Mellanby Memorial Lecture for 1981.

REFERENCES

1. Aikawa, M. and T.M. Seed (1980). Morphology of Plasmodia. In: *Malaria. v. 1.* Ed. J.P. Kreier. (Academic Press, New York) : 284-344.
2. Alving, C.R., I. Schneider, G.M. Swartz and E.A. Steck (1979). Sporozoite-induced malaria : therapeutic effects of glycolipids in liposomes. *Science, N.Y.*, **205:** 1142-1144.
3. Bass, C.C. and F.M. Johns (1912). The cultivation of malarial plasmodia. *(Plasmodium vivax* and *Plasmodium falciparum) in vitro. J. Exp. Med.*, **16:** 567-579.
4. Beaudoin, R.L. and M. Aikawa (1968). Primaquine-induced changes in morphology of exoerythrocytic stages of malaria. *Science, N.Y.*, **160:** 1233-1234.
5. Bennett, T.P. and W. Trager (1967). Pantothenic acid metabolism during avian malaria infection : Pantothenate kinase activity in duck erythrocytes and in *Plasmodium lophurae. J. Protozool.*, **14:** 214-216.
6. Bjorkman, A., J. Brohult, V. Sirleaf, M. Willcox and E. Bengtsson (1980). *Plasmodium falciparum* resistance to pyrimethamine and chloroproguanil—host or parasite dependent. *Ann. Trop. Med. Parasitol.*, **74:** 245-248.
7. Brand, T. Von (1973). *Biochemistry of Parasites.* (Academic Press, London, New York).
8. Canfield, C.J. and R.S. Rozman (1974). Clinical testing of new antimalarial compounds. *Bull. WHO,* **50:** 203-212.
9. Center for Disease Control (CDC) (1980). *Plasmodium falciparum* malaria contracted in Thailand resistant to chloroquine and sulfonamide-pyrimethamine. *Illinois Morbid. Mortal. Wkly. Rep.*, **29:** 493-495.
10. Chern, C.J. and E. Beutler (1975). Pyridoxal kinase; Decreased activity in red blood cells of Afro-Americans. *Science, N.Y.*, **187:** 1084-1086.
11. Chou, A.C., R. Chevli and C.D. Fitch (1980). Ferriprotoporphyrin IX fulfils the criteria for identification as the chloroquine receptor of malaria parasites. *Biochemistry*, **19:** 1543-1549.
12. Clark, I.A., A.C. Allison and F.E.G. Cox (1976). Protection of mice against *Babesia* and *Plasmodium* with BCG. *Nature*, **259:** 309-311.
13. Clark, I.A., F.E.G. Cox and A.C. Allison (1977). Protection of mice against *Babesia* spp. and *Plasmodium* spp. with killed *Corynebacterium parvum. Parasitology*, **74:** 9-18.
14. Cochrane, A.H., R.S. Nussenzweig and E.H. Nardin (1980). Immunization against sporozoites. In *Malaria v. 3.* Ed. J.P. Kreier. (Academic Press, New York): 163-202.
15. Cohen, S. (1979). Immunity to malaria. *Proc. R. Soc. B. Lond.*, **203:** 323-345.
16. Cohen, S. and G.A. Butcher (1971). Serum antibody in acquired malarial immunity. *Trans. R. Soc. Trop. Med. Hyg.*, **65:** 125-135.
17. Cox, F.E.G. (1978). Specific and non-specific immunisation against parasitic infections. *Nature. Lond.*, **273:** 623-626.
18. Dvorak, J.A., L.H. Miller, W.C. Whitehouse and T. Shiroishi (1975). Invasion of erythrocytes by malarial merozoites. *Science, N.Y.*, **187:** 748-750.
19. Ferone, R. (1977). Folate metabolism in malaria. *Bull. WHO*, **55:** 291-298.
20. Ferone, R., J.J. Burchall and B.H. Hitchings (1969). *Plasmodium berghei* dihydrofolate reductase. Isolation, properties and inhibition by antifolates. *Mol. Pharmacol.*, **5:** 49-59.
21. Fulton, J.D. and T.H. Flewitt (1955). Electron microscope studies in malaria. *Trans. R. Soc. Trop. Med Hyg.*, **49:** 302.
22. Gilles, H.M. and D.F. Clyde (1974). Acetylator phenotype in sulphonamide resistant falciparum malaria. *Ann. Trop. Med. Parasitol.*, **68:** 367-368.
23. Gillet, J., P.J. Jacques and F. Herman (1980). Particulate β 1-3 glucan and causal prophylaxis of mouse malaria. In *Macrophages and Lymphocytes,*

functions and interactions. Eds. M.R. Escobar and H. Friedman. (Plenum Press, New York): 307–313.

24. Greenwood, B.M., A.M. Bradley-Moore, A. Palit and A.D.M. Bryceson (1972). Immunosuppression in children with malaria. *Lancet*, **1**: 169–172.
25. Greenwood, B.M., H.C. Whittle and D.H. Molyneux (1973). Immunosuppression in Gambian trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.*, **67**: 846–850.
26. Greenwood, B.M. and R.M. Vick (1975). Evidence for a malaria mitogen in human malaria. *Nature*, **257**: 592–594.
27. Howells, R.E., W. Peters and J. Fullard (1970). The chemotherapy of rodent malaria, XIII. Fine structural changes observed in the erythrocytic stages of *Plasmodium berghei* following exposure to primaquine and menoctone. *Ann. Trop. Med. Parasitol.*, **64**: 203–207.
28. Howells, R.E., W. Peters and C.A. Homewood (1972). Physiological adaptability of malaria parasites. In *Comparative biochemistry of parasites*. Ed. H. van den Bossche. (Academic Press, New York, London): 235–258.
29. Howells, R.E. and L. Maxwell (1973). Citric acid cycle activity and chloroquine resistance in rodent malaria parasites: the role of the reticulocyte. *Ann. Trop. Med. Parasitol.*, **67**: 285–300.
30. Huff, C.G., A.B. Weathersby, A.C. Pipkin and G.H. Algire (1960). The growth of exoerythrocytic stages of avian malaria within diffusion chambers in different hosts. *Exp. Parasitol.*, **9**: 98–104.
31. Kilejian, A. and J.B. Jensen (1977). A histidine-rich protein from *Plasmodium falciparum* and its interaction with membranes. *Bull. WHO*, **55**: 191–197.
32. Kreier, J.P. and T.J. Green (1980). The vertebrate host's immune response to plasmodia. In *Malaria v. 3*. Ed. J.P. Kreier. (Academic Press, New York): 111–162.
33. Krotoski, W.A., D.M. Krotoski, P.C.C. Garnham, R.S. Bray, R. Killick-Kendrick, C.C. Draper, G.A.T. Targett and M.W. Guy (1980). Relapses in primate malaria; discovery of two populations of exoerythrocytic stages. Preliminary note. *Br. Med. J.*, **1**: 153–154.
34. Macdonald, G. (1957). Community aspects of immunity to malaria. *Br. Med. Bull.*, **8**: 33–36.
35. Macdonald, G. (1957). *The epidemiology and control of malaria*. (Oxford University Press, London, New York, Toronto).
36. McGregor, I.A. (1979). Basic considerations concerning field trials of malaria vaccines in human populations. *Bull. WHO*, **57** (Suppl.): 267–271.
37. Miller, L.H., S.J. Mason, M. Dvorak, H. McGinnis and I.K. Rothman (1975). Erythrocyte receptors for (*Plasmodium knowlesi*) malaria : Duffy blood group determinants. *Science, N.Y.*, **189**: 561–562.
38. Nussenzweig, R.S., A.H. Cochrane and H.J. Lustig (1978). Immunological responses. In *Rodent malaria*. Eds. R. Killick-Kendrick and W. Peters. (Academic Press, London, New York, San Francisco): 247–307.
39. Peters, W. (1968). The chemotherapy of rodent malaria, VII. The action of some sulphonamides alone or with folic reductase inhibitors against malaria vectors and parasites. Part 2. Schizontocidal action in the albino mouse. *Ann. Trop. Med. Parasitol.*, **62**: 488–494.
40. Peters, W. (1970). *Chemotherapy and drug resistance in malaria*. (Academic Press, London, New York).
41. Peters, W., J.H. Portus and B.L. Robinson (1973). The chemotherapy of rodent malaria, XVII. Dynamics of drug resistance. Part 3: Influence of drug combinations on the development of resistance to chloroquine in *P. berghei*. *Ann. Trop. Med. Parasitol.*, **67**: 143–157.
42. Peters, W. (1974a). Prevention of drug resistance in rodent malaria by the use of drug mixtures. *Bull. WHO*, **51**: 379–383.
43. Peters, W. (1974b). Possible new antimalarial drugs. *Nature*, **204**: 305–306.
44. Peters, W. (1980a). Chemotherapy of malaria. In *Malaria v. 1*. Ed. J.P. Kreier. (Academic Press, New York): 145–283.
45. Peters, W. (1980b). Interactions entre *Plasmodium* et les antipaludiques. *Cah. ORSTOM Ser. Ent. Med. Et Parasitol.*, **18**: 121–125.
46. Peters, W. (1981). The interaction of drugs and Immunity in malaria. *Indian J. Malariol.*, **20**(2): 93–106.
47. Pirson, P., R.F. Steiger, A. Trouet, J. Gillet and F. Herman (1980). Primaquine liposomes in the chemotherapy of experimental murine malaria. *Ann. Trop. Med. Parasitol.*, **74**: 383–391.
48. Rao, R. and M. Sirsi (1956). Avian malaria and B-complex vitamins. II. Riboflavin. *J. Ind. Inst. Sci.*, **38**: 186–189.
49. Ray, A.P. (1977). Malaria control, achievements, problems and prospects of eradication. *J. Com. Dis.*, **9**: 145–171.
50. Ray, A.P. (1979). Some aspects of *P. falciparum* containment programme. *Indian J. Med. Res.*, **70** (Suppl.): 1–13.
51. Rieckmann, K.H. (1967). A new repository antimalarial agent, CI-564, used in a field trial in New Guinea. *Trans. R. Soc. Trop. Med. Hyg.*, **61**: 189–198.
52. Rozman, R.S. and C.J. Canfield (1979). New experimental antimalarial drugs. *Adv. Pharmacol. Chemother.*, **16**: 1–43.
53. Salaman, M.H., N. Weddercourn and L.J. Bruce-Chwatt (1969). The immuno-depressive effect of

murine *Plasmodium* and its interaction with murine oncogenic viruses. *J. Gen. Microbiol.*, **59**: 383–391.

54. Sherman, I. (1979). Biochemistry of *Plasmodium* (malariel parasites). *Microbiol. Rev.*, **43**: 453–495.

55. Siddiqui, W.A. and W. Trager (1964). Comparative bioautography of folic and folinic acids of erythrocytes and livers of normal ducks and ducks infected with malarial parasites. *J. Parasitol.*, **50**: 753–756.

56. Sinden, R.E. (1978). Cell biology. In *Rodent malaria*. Eds. R. Killick-Kendrick and W. Peters. (Academic Press, London, New York, San Francisco): 85–168.

57. Singh, Jaswant (1955). Some problems on chemotherapy of malaria. *Indian J. Malariol.*, **9**: 271–275.

58. Sinton, J.A. (1955). Some lacunae in our knowledge of the malaria parasite. *Indian J. Malariol.*, **9**: 229–245.

59. Skelton, F.S., R.S. Pardini, J.C. Heidker and K. Folkers (1968). Inhibition of coenzyme Q systems by chloroquine and other antimalarials. *J. Am. Chem. Soc.*, **90**: 5334–5336.

60. Skelton, F.S., K.D. Lunan, K. Fokers, J.V. Schnell, W.A. Siddiqui and Q.M. Geiman (1969). Biosynthesis of ubiquinones by malarial parasites. I. Isolation of (^{14}C) ubiquinones from cultures of rhesus monkey blood infected with *Plasmodium knowlesi*. *Biochemistry*, **8**: 1284–1287.

61. Smith, J.E. and R.E. Sinden (1981). Studies on the uptake of sporozoites of *P. yoelii nigeriensis* by perfused rat liver. *Trans. R. Soc. Trop. Med. Hyg.*, **75**: 188–189.

62. Taylor, A.E.R. and J.R. Baker (1978). *Methods of cultivation parasites in vitro*. (Academic Press, London, New York, San Francisco).

63. Terzian, L.A., P.A. Ward and N. Stahler (1951). A new criterion for the selection of compounds for curative activity in *Plasmodium vivax* malaria. *Am. J. Trop. Med.*, **31**: 692–697.

64. Thong, Y.H. and A. Ferrante (1980). Immunopotentiation by pyrimethamine in the mouse. *Clin Exp. Immunol.*, **39**: 190–194.

65. Thong, Y.H., A. Ferrante and B. Rowan-Kelly (1978). Primaquine inhibits mitogen-induced human lymphocyte proliferative responses. *Trans. R. Soc. Trop. Med. Hyg.*, **72**: 537–539.

66. Trager, W. (1977). Cofactors and vitamins in the metabolism of malarial parasites. Factors other than folates. *Bull. WHO*, **55**: 285–289.

67. Trager, W. and J.B. Jensen (1976). Human malaria parasites in continuous culture. *Science, N.Y.*, **193**: 673–675.

68. Verhave, J.P., J.H.E. Th. Meuwissen and J. Golenser (1980). The dual role of macrophages in the sporozoite-induced malaria infection. A hypothesis. *Int. J. Nucl. Med. Biol.*, **7**: 149–156.

69. Warhurst, D.C. and S.C. Thomas (1975). Localization of mepacrine in *Plasmodium berghei* and *Plasmodium falciparum* by fluorescence microscopy. *Ann. Trop. Med. Parasitol.*, **69**: 417–420.

70. Wise, D.L., J.D. Gresser and G.J. McCormick (1979). Sustained release of dual antimalarial system. *J. Pharm. Pharmacol.*, **31**: 201–204.

Indian Journal of Malariology
Vol. 21, June 1984, pp. 17-20.

Effect of Chloroquine Treatment on Complement Levels in *Plasmodium knowlesi* Infected Rhesus Monkeys

R.N. PRASAD[1], N.K. GANGULY[1], R.C. MAHAJAN[1] and S.K. GARG[1]

C_3 and CH_{50} levels in Rhesus monkeys have been estimated during acute *Plasmodium knowlesi* infection in Rhesus monkeys and following treatment with chloroquine at different levels of parasitaemia. The depletion in C_3 and CH_{50} levels correlated with the level of parasitaemia. The chloroquine treatment reversed the trend in animals treated at less than 30 percent of parasitaemia and there was recovery in the level of C_3 and CH_{50} by the 9th post drug-administration day. The probable mechanism of reversibility by chloroquine in their levels in infected and treated animals is discussed.

INTRODUCTION

Lower levels of total complement activity have been well documented during malaria infection in mice (Krettli *et al.*, 1976; June *et al.*, 1979), monkeys (Fogel *et al.*, 1966; Cooper and Fogel, 1966) and human beings (Greenwood and Brueton, 1974; Adam *et al.*, 1981). Recently, Ward *et al.*, (1981), observed that *P. berghei* infection was much more severe and even fatal in C_3 depleted rats than C_3 intact (control) ones, suggesting a role of complement in the immune status of the host. Since depletion in complement components has been shown to correlate inversely with the schizont rupture (Atkinson *et al.*, 1975), it is important to know what happens when the parasites are eliminated from the host after drug-treatment at different levels of parasitaemia and how long the hypocomplementaemia persists. To the best of our knowledge, little is known so far in this direction. Therefore, the present study was undertaken to see the effect of drug-treatment on C_3 and CH_{50} levels at different levels of parasitaemia in *P. knowlesi* infected Rhesus monkeys.

Accepted for publication: 12 April 1984.
[1]Department of Parasitology and Pharmacology
Postgraduate Institute of Medical Education and Research
Chandigarh-160 012.

MATERIAL AND METHODS

Rhesus monkeys and mode of infection: Twelve normal, healthy, tuberculin negative, rhesus monkeys, weighing 4 to 5 kg and without any microbial infection, were included in this study. All the animals were challenged with 10×10^5 *P.knowlesi* infected erythrocytes. Parasitaemia was monitored daily by peripheral blood-smear examination. This was expressed as percent of parasitaemia. The animals were divided into 4 groups of 3 monkeys each.

Group I: The animals in this group were followed up for higher levels of parasitaemia (more than 60 percent) and no treatment was given.

Group II, III and IV: Animals were treated with 20 mg chloroquine (orally) per kg body weight for seven days at 8-10, 20-30 and more than 60 percent of parasitaemia respectively (Schmidt, 1978).

Estimation of C_3 and CH_{50} levels: 5 ml of clotted blood was taken from each animal starting from pre-infection period, at different levels of parasitaemia and during drug treatment. Serum was separated and stored at — 70°C until use.

C_3 level was estimated by the method of Mancini *et al.* (1965), as described by Ganguly *et al.* (1977), for rhesus monkeys. The value was expressed as mg percent.

The total haemolytic activity (CH_{50}) was assayed (Ganguly *et al.*, 1979). The value was calculated as units per ml.

RESULTS

Effect of parasitaemia on C_3 and CH_{50} levels: The pre-infection mean levels of C_3 and CH_{50} in group I monkeys were 168.66±18.20 mg/100 ml and 31.42±2.14 µ/ml, respectively. At 8-10 percent parasitaemia, the respective levels dropped to 103.00±7.87 and 22.58±1.85. They declined significantly ($P<0.001$) to 50.33±3.29 and 9.6±0.97 respectively, when the parasitaemia was more than 60 percent. The degree of parasitaemia correlated with the decrease in the levels of C_3 and CH_{50} ($r=0.891$ and -0.921, respectively).

Effect of chloroquine therapy: The decrease in C_3 and CH_{50} levels in *P. knowlesi* infected monkeys of group II, III and IV showed a similar trend as in group I animals as long as they remained untreated. The chloroquine treatment in these groups of animals at different levels of parasitaemia inhibited the further decrease in the levels of C_3 and CH_{50}, respectively (Fig. 1 and 2).

In group II, III and IV animals, at the time of treatment, when the parasitaemia was 8-10, 20-30 and more than 60 percent, the levels of C_3 and CH_{50} were 114.66±19.39 and 22.70±2.17, 71.33±10.20 and 15.96±1.62 and 58.33±3.39 and 10.73±0.89, respectively. After chloroquine therapy, the parasites disappeared from the peripheral blood and the levels of C_3 and CH_{50} recovered by the 7th to the 9th day of treatment in group II and III animals (the value

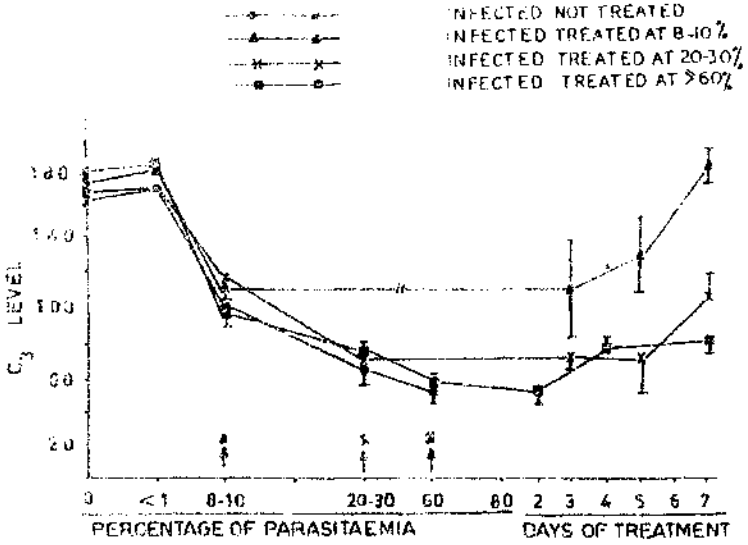


Fig. 1. C_3 levels in infected and treated Rhesus monkeys. (Arrows indicate first dose of drug-administration in corresponding group).

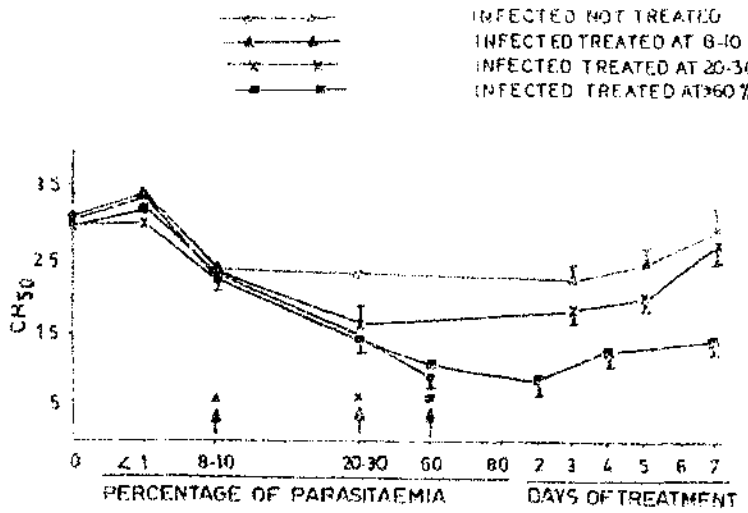


Fig. 2. CH_{50} levels in infected and treated Rhesus monkeys. (Arrows indicate first dose of drug administration in corresponding group).

being 184.00±9.09 and 30.10±3.16 and 173.66±14.65 and 31.96±1.91 of C_3 and CH_{50} respectively with $P>0.05$). The mean values of C_3 and CH_{50} in group III was lower (103.00±7.87 and 27.27±2.37) than in group II animals (184.00±0.09 and 30.10±3.16). On the 7th day after the drug treatment, the difference in the mean value of C_3 and CH_{50} in group IV was statistically significant ($P<0.01$ when compared with the pre-infection values.

DISCUSSION

These results show that there was a decrease in serum C_3 and CH_{50} levels during the course of *P. knowlesi* infection in Rhesus monkeys and this correlated with the level of parasitaemia. Similar observations have also been made by Fogel *et al.* (1966). The hypocomplementaemia occurs either due to the activation of the classical (Greenwood and Brueton, 1974) and alternate (Adam *et al.*, 1981) pathways or due to utilization of complement by the parasites (Cooper and Fogel, 1967; Adam *et al.*, 1981). The activation of complement is mainly by immune complexes (Adam *et al.*, 1981). Malaria antigen itself can activate the complement pathway (Adam *et al.*, 1981). In malaria it has been shown that serum Ca^{++} and Mg^{++} are increased (Gupta *et al.*, 1982) which can exaggerate the activation of the complement pathway (Levin *et al.*, 1953; Mayer, 1946).

Chloroquine treatment in different groups of monkeys at different levels of parasitaemia inhibited the further decrease in the level of C_3 and CH_{50}. Group II animals treated at a lower (8-10 percent) level of parasitaemia showed a rapid recovery in their C_3 and CH_{50} levels by 7th day post-treatment while the animals treated at a higher level of parasitaemia (30 percent) recovered after 9 days. Ree (1976), also, has reported the recovery in C_3 and CH_{50} levels in patients where the parasitaemia is generally very low after chloroquine treatment but the above investigators did not report the effect of antimalarial treatment on the complement levels of hosts treated at different levels of parasitaemia. The recovery in C_3 and CH_{50} levels, after chloroquine treatment is probably due to the disappearance of the parasite and its antigen (June *et al.*, 1979), dissociation of immuno-complexes (Beneze and Johnson, 1965) or loss of anti-complementary activity, of such immunocomplexes (Onyewoter, 1978) by the drug. Chloroquine also can bind and lower the

level of calcium and magnesium ions (Neblet *et al.*, 1965), which might be inhibiting the activation of the complement. Moreover, the complement biosynthesis may be increased during injury or infection (Cooper and Fogel, 1967; Schur and Austen, 1968). However, the recovery in C_3 and CH_{50} level in groups III and IV (treated at 20-30 and more than 60 percent of parasitaemia, respectively) was delayed and remained unrecoverable in the latter group during the study period. It was probably due to severe hypocomplementaemia at higher levels of parasitaemia. At this stage, the highly affected liver (Krettli *et al.*, 1976) and monocytes (Nelson, 1976) might be synthesising the complement at a very low rate.

ACKNOWLEDGEMENTS

The authors are grateful to the Indian Drugs and Pharmaceuticals Limited, India, for financial support and May and Baker (India) Limited, Bombay, for the supply of chloroquine sulphate.

REFERENCES

1. Adam, C.M., M. Geniteau, M. Gougerot-Pocidalo P. Verroust, J. Lebras, C. Gibert and L. Morel-Maroger (1981). Cryoglobulins circulating immune complexes and complement activation in cerebral malaria. *Infect. Immun.*, **31:** 530-535.
2. Atkinson, J.P., R.H. Glew, F.A. Neva and M.M. Frank (1975). Serum complement and immunity in experimental simian malaria. *J. Infect. Dis.*, **131:** 26-33.
3. Beneze, G. and G.D. Johnson (1965). Inhibition of antinuclear factor reaction by chloroquine. *Immunology*, **9:** 201-204.
4. Cooper, N.R. and B.J. Fogel (1966). Complement in acute experimental malaria II – Alteration in complement component. *Milit. Med.*, **131** (Suppl.): 1180-1190.
5. Cooper, N.R. and B.J. Fogel (1967). Complement in normal and diseased person. *J. Pediatr.*, **70:** 982-996.
6. Fogel, B.J., A.E. Von Doonhoff, N.R. Cooper and E.H. Fife (1966). Complement in acute experimental malaria I–Total haemolytic activity. *Milit. Med.*, **131:** 1173-1179.
7. Ganguly, N.K., K.S. Chugh, Y. Pal and R.P. Sapru (1977). Comparative value of C_3 in normal rhesus monkeys and man. *Indian J. Med. Res.*, **66:** 570-575.
8. Ganguly, N.K., D. Arya and N.L. Chitkara (1979). Comparative evaluation of CH_{50} titration methods. *Indian J. Pathol. Microbiol.*, **20:** 111-116.
9. Greenwood, B.M. and M.J. Brueton (1974). Complement activation in children with acute malaria. *Clin. Exp. Immunol.*, **18:** 267-272.
10. Gupta, C.M., A. Alam, P.N. Mathur and G.P. Dutta (1982). A new look at non-parasitized red cells of malaria infected monkeys. *Nature*, **299:** 259-260.
11. June, C.H., C.E. Contreras, L.H. Parrin, P.H. Lambert and P.A. Miescher (1979). Circulating and tissue bound immunocomplex formation in murine malaria. *J. Immunol.*, **122:** 2154-2157.
12. Krettli, A.U., V. Nussenzweig and R.S. Nussenzweig (1976). Complement alteration in rodent malaria. *Am. J. Trop. Med. Hyg.*, **25:** 34-41.
13. Levine, L., A.G. Osler and M.M. Mayer (1953) The respective role of Ca^{++} and Mg^{++} in immune haemolysis. *J. Immunol.*, **71:** 374-379.
14. Mancini, G., A.O. Garbenera and J.F. Hereman (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochem.*, **2:** 235-242.
15. Mayer, M.M. (1946). The activating effect of Magnesium and other cations on the haemolytic function of complement. *J. Exp. Med.*, **84:** 535-548.
16. Mayer, M.M. (1961). In *Experimental Immunochemistry*. (2nd edition). Ed. E.A. Kabat and M.M. Mayer. (Charles, C. Thomas, Springfield, Illinois): 134.
17. Neblet, T.T., T.K. Burnham and N. Kay (1965). Chloroquine-its mechanism of action upon immune phenomena. *Arch. Dermatol.*, **92:** 720-725.
18. Nelson, D.S. (1976). *Immunobiology of the Macrophages*. (Academic Press, New York, London).
19. Onyewoter, I.I. (1978). Immunocomplexes in healthy Nigerians *Trans. R. Soc. Trop. Med. Hyg.*, **72:** 386-388.
20. Ree, G.H. (1976). Complement and malaria. *Ann. Trop. Med. Parasitol.* **70:** 247-248.
21. Schmidt, L.H. (1978). *Plasmodium falciparum* and *Plasmodium vivax* infection in owl monkey (*Aotus trivirgates*). *Am. J. Trop. Med. Hyg.*, **27:** 703-709.
22. Schur, P.H. and K.F. Austen (1968). Complement in human disease. *Annual Rev. Med.*, **19:** 1-24.
23. Ward, P.A., R.B. Sterzel, H.L. Lucia, G.H. Campbell and R.M. Jack (1981). Complement does not facilitate plasmodial infection. *J. Immunol.*, **126:** 1826.

Indian Journal of Malariology
Vol. 21, June 1984, pp. 21-24

Results of 3-Day Radical Treatment of *Plasmodium vivax* in North Arcot and South Arcot Districts of Tamil Nadu

N.C. APPAVOO[1], R.G. ROY[2] and V. KAPALI[3]

The adult dose prescribed for *P. vivax* under the National Malaria Eradication Programme (NMEP) of India amounts to 600 mg chloroquine base and 75 mg of primaquine base spread over 5 days for radical treatment. In a study conducted in 1978-1980 in two districts of Tamil Nadu, the total dose of chloroquine and primaquine was kept unchanged but the duration of the radical treatment was reduced from 5 days to 3 days (30 mg+30 mg+15 mg). The revised schedule was well tolerated and the overall breakthrough rate for 1203 patients on one year follow up was only 3.76%. In the group of 918 patients in South Arcot District, the rate was only 1.37%, but in the North Arcot District, it was higher (18.03%). The reason for this difference is not clear, but as the latter area received only focal spray, it could be partly due to higher transmission. The overall failure rate of only 3.76% compared well with the standard 5-day course followed in the Indian programme. It gave the much needed operational advantage of reduced need for manpower.

INTRODUCTION

In the National Malaria Eradication Programme (NMEP) of India, all fever cases are given a presumptive treatment (PT) of 600 mg base of Chloroquine. Should the slide show *P. vivax* parasites, an anti-relapse radical treatment (RT) is given at which time the chloroquine dose (600 mg base) is again repeated, and in addition, primaquine 15 mg base (adult) is given daily for 5 days. The regimen is being adopted from 1960 and was kept unchanged at the time of introduction of the modified plan of operations in the NMEP in 1977 (Pattanayak and Roy, 1980).

Accepted for publication: 24 April 1984.
[1]Directorate of Public Health and Preventive Medicine, No. 259, Anna Salai, Madras 600006, Tamil Nadu.
[2]Central Leprosy Teaching and Research Institute, Chengalpattu 603001, Tamil Nadu.

By the time the health worker goes to the patient for RT, he bcomes afebrile due to the PT given earlier and does not see the need for RT and hence full co-operation is not received. If the period of RT can be reduced without any compromise on efficacy and side effects, it would offer a great operational advantage and, if needed, a larger number of patients could be covered by RT.

The results of a trial with 3-day RT course as observed in the districts of North Arcot and South Arcot are presented here.

MATERIAL AND METHODS

The study was conducted during 1978-80 in three Primary Health Centres (PHCs) of North Arcot and 13 PHCs in South Arcot District. The approximate population was 2,70,000 and 13,00,000 respectively. The other PHCs of these

two districts either had no malaria, or very few imported cases not available for follow-up, or had moderately heavy transmission of malaria making the area unsuitable for a study of relapse phenomenon due to vivax malaria.

No comparison area was kept for concurrently studying the results of 5-day course since 3-day Radical Treatment course was adopted all over Tamil Nadu and the results were compared with the earlier published work.

No pregnant woman or infant or acutely ill person was taken up for this study.

Existing system of giving 600 mg chloroquine base each, at the time of PT and first day of RT continued. But the primaquine dose was changed to 30 mg base on day 0 and day 1 and 15 mg on day 2 (adult dose). Children received proportionately smaller doses i.e. those of 1-4 years, 5-8 years and 9-14 years received 1/4, 1/2 and 3/4 of the adult dose, respectively. The gap between the PT and RT was generally within a period of 7-10 days.

As practised under NMEP, blood smears were taken on day 7 and thereafter, at monthly intervals for 12 months and one hundred thick smears were examined, irrespective of the fact whether the individual is febrile or not, though the results have been presented here on a quarterly basis. The breakthrough cases were re-treated with the same dose and the same schedule.

A close watch was kept to note down adverse side-effects of the modified schedule.

The insecticidal spray instructions of NMEP, as per the modified plan of operations were followed. All the three Primary Health Centres of North Arcot district had API less than 2 and qualified for focal spray only around the positive cases (i.e. not regular spray in the entire locality). In South Arcot district, out of 13 PHCs under study, 10 had API below 2 and, therefore, received only focal spray. But the remaining three PHCs were given three rounds of malathion spray, as the API was above 2 due to some local malaria transmission. The vector, *A. culicifacies*, was resistant to DDT/HCH in these PHCs. These steps were helpful in considerably reducing the transmission, though it was not totally eliminated.

The term 'breakthrough' has been used for any case where the parasites of vivax species reappeared after the initial clearance and does not refer to only relapse cases.

RESULTS AND DISCUSSION

Results are presented in Table I. A total of 1345 *P. vivax* cases were detected, of which 1203 (89.4%) were available for RT. Of the persons given RT, only 425 (35.3%) could be followed up till the end of one year. Eight out of 713; 5 out of 629; 3 out of 501 and none out of 425 showed breakthrough during I, II, III and IV quarter respectively.

As already pointed out by Roy *et al.* (1977), it was rather difficult to work out the breakthrough rate, as the number followed up was not constant and gradually declined. By taking the number followed up at the time of the 12th follow-up, the overall rate works out as 3.76%.

Immediate results of RT were very good as all the persons were negative. There was no manifest adverse reaction to the drug regimen and it was operationally very convenient to the field staff as well as to the patients.

From 1977, till the time of writing this note, more than 3,00,000 vivax cases have been treated in Tamil Nadu with revised schedule and no manifest adverse reaction was noted; there was no death attributable to RT; of course, no

Table 1. Results of 3-day course of Radical Treatment in *P. vivax* with 1200 mg chloroquine and 75 mg Primaquine (both base) (1978–80)

District	Cases detected	Given RT	Results of follow up (No.positive/No.followed)					Percent break-through (%)
			I Qr.	II Qr.	III Qr.	IV Qr.	For 1 year	
1. North Arcot 3 blocks	303	285	7/135 (6 in 2nd and 1 in 3rd month)	2/119 (1 each in 5th & 6th month)	2/92 (1 in 7th and 9th month)	0/61	11/61	18.03
2. South Arcot 13 blocks	1042	918	1/578 (in 3rd month)	3/510 (1 in 4th & 2 in 6th month)	1/409 (in 7th month)	0/364	5/364	1.37
Total	1345	1203	8/713	5/629	3/501	0/425	16/425	3.76

Note: 1. On day 7, all the persons were negative.
2. All the 16 break-through cases were re-treated with the same 3-day course; No further recurrence noted during the period of follow-up.

haematological investigation was done for minor haemolysis.

Though the 5-day regimen is in vogue in the NMEP of India from 1960, there are only a few published records about the efficacy in different parts of the country. The original work which formed the basis of this Indian regimen was carried out by Basavaraj(1960) in 1958-59 in Karnataka. During a follow-up period of 678 cases for 18 months, he found a breakthrough rate of 6.03%.

In the Ratlam NMEP Unit of Madhya Pradesh in 1973, the breakthrough rate was found to be 8.4% during one year follow-up (Sharma *et al.*, 1973) in a group of 130 *P. vivax* cases.

In Tamil Nadu, more extensive work was carried out. Roy *et al.* (1979), in 1974 and 1975 studied the results of 5-day RT of 8329 vivax cases in Dharmapuri district and recorded a breakthrough rate of only 1.28%.

In the present study, out of 1203 given RT, only 425(35.3%) could be followed up for one year. In North Arcot, out of 285 given RT, 11 cases showed breakthrough. On the basis of the small number last followedup (i.e. 61), the rate comes to 18.03%; the maximum number (7) was during the first quarter.

In the South Arcot district, out of 918 given RT, only 5 cases showed breakthrough. The rate works out as 1.37%.

The reason for this great disparity in two adjoining districts is not clear. As already stated, in the 3 PHCs of North Arcot district—only focal spray was given, as the API was low. But it also had some areas of intense malaria transmission in the Thenpennai river belt and it is a distinct possibility that some of the patients had intra-district movement, but in the absence of epidemiological investigation, no conclusion could be drawn regarding whether it was due to treatment failure or reinfection or some other undetected factor.

By and large, the 3-day regimen compares well with the standard 5-day course given in other parts of India.

Similar studies should be carried out in other States also in properly selected areas. If the findings are similar, it would reduce the load of field work considerably. Besides, it is also necessary to know how the drug, primaquine, is working on *P. vivax* after more than 20 years of drug pressure.

ACKNOWLEDGEMENTS

The authors are indebted to all the field and laboratory staff and the District Malaria Officers' for their sincere co-operation.

REFERENCES

1. Basavaraj, H.R. (1960). Observations on the treatment of 678 malaria cases with primaquine in an area free from malaria transmission in Mysore State. *Indian J. Malariol.*, **14**: 269.
2. Pattanayak, S. and R.G. Roy (1980). Malaria in India and modified plan of operations for its control. *J. Com. Dis.*, **12**(1): 1-14.
3. Roy, R.G., K.P. Chakrapani, D. Dhingaran, N.L. Sitaraman and R.B. Ghose (1977). Efficacy of 5-day radical treatment of *P. vivax* infection in Tamil Nadu. *Indian J. Med. Res.*, **65**(5): 652-656.
4. Roy, R.G., C.A.K. Shanmugham, K.P. Chakrapani and A.V. Ganesan (1979). Results of 5-day course of radical treatment of *Plasmodium vivax* in six districts of Tamil Nadu. *Indian. J. Med. Res.*, **69**(6): 939-943.
5. Sharma, M.I.D., P.N. Sehgal, B.K. Vaid, R.C. Dubey, S. Nagendra, P.K. Paithmo and M.L. Joshi (1973). Effectiveness of drug schedule being followed under NMEP India for radical cure of vivax malaria cases. *J. Com. Dis.*, **5**(4): 167-174.

Indian Journal of Malariology
Vol. 21, June 1984, pp. 25-30.

Chloroquine Resistance of *Plasmodium falciparum* in Sonapur PHC Area (Assam) Detected by Micro *in vitro* and *in vivo* Tests

A.N. RAICHOWDHURI[1], S. SINHA[1], BHUYYA[2], A. GAJANANA[1] and K.K. BAISHYA[3]

Sensitivity of *Plasmodium falciparum* to chloroquine in Sonapur PHC Area (Assam) was assessed by WHO 28-day extended *in vivo* test and micro *in vitro* test in pre-dosed NICD plates using sealing technique and in WHO plates using candle jar method. Resistance was recorded in 13/20 (65%) patients *in vivo* and 4/7 (59%) *in vitro*. Results of *in vivo* and *in vitro* tests were in agreement in 6/7 cases. Linear regressions were fitted separately for responses in NICD and WHO plates, and there was no statistically significant difference between the regression co-efficients of the two, indicating that the two methods were comparable. The present study clearly indicated the value of micro *in vitro* tests and identified chloroquine resistance in the study-site which calls for a revised drug policy for Sonapur PHC area.

INTRODUCTION

Resistance of *Plasmodium falciparum* to chloroquine in some parts of Assam has been reported earlier (Sehgal *et al.*, 1973; Pattanayak *et al.*, 1979). Micro *in vitro* test developed by Rieckmann *et al.* (1978) has been adapted to monitor susceptibility of field isolates of *P. falciparum* to 4-aminoquinolines (WHO Monograph Series, 1981), and is undergoing extensive field evaluation. This test was further modified by introducing a sealing technique instead of using a candle jar in the test (Gajanana *et al.*, 1982). A study was carried out to assess the sensitivity of *P. falciparum* to chloroquine in Sonapur PHC Area (Kamrup District) of Assam by micro *in vitro* test employing the WHO and the NICD methods and compare the results with responses *in vivo*.

MATERIAL AND METHODS

Study-site and selection of patients: The investigation was done in 15 villages of the PHC Area which is covered under modified plan of operation of the NMEP. Epidemiological data for 1981 of the PHC were ABER 26.13, API 23.12, SPR 8.84 and SFR 7.47. Although falciparum cases are recorded year round, the peak transmission season is between May and

Accepted for publication: 24 April 1984.
[1]National Institute of Communicable Diseases
22-Sham Nath Marg, Delhi 110054.
[2]ICMR Monitoring Team
National Malaria Eradication Programme, Shillong.
[3]Directorate of Health Services, Assam.

July. Therefore, the study was conducted during December, 1982, in order to minimise the chances of re-infections during the *in vivo* extended tests. Thick blood smears were made from all fever cases by house-to-house visits and examined after staining with Giemsa's stain. Patients with only *P. falciparum* were selected for studies, after ensuring the absence of 4-aminoquinolines in their urine by the Dill and Glazko test.

In vivo tests: In the WHO 28-day extended field test, an adult (14 years and above) received 600 mg of chloroquine base on day 0 and D-1 followed by 300 mg on D-2. Children received proportionately smaller doses depending upon the age. Absorption of the drug was confirmed by the urine test on D-3. Asexual parasites on D-0, D-3, D-7, D-14 and D-28 were counted on Giemsa stained thick smears and expressed as Bruce-Chwatt's Parasite Density Index (PDI) (Bruce-Chwatt, 1980).

Micro in vitro tests: Tests were carried out in pre-dosed plates supplied in the WHO Kit (Lot No. 00461514 charged on 15th September, 1982 and 00381514 charged on 7th June, 1982) as well as in pre-dosed plates prepared by the NICD. In each plate, wells A to H contained 0, 1, 2, 4, 5, 7, 8, 16 and 32 pmols of chloroquine base respectively.

From individual patients, approximately 100 µl of blood was aseptically collected in heparin (10 i.u./ml of blood) and after diluting 9 times with RPMI 1640 growth medium without serum, 50 ul of the suspension was added to each well from A to H. Two incubation procedures were followed. NICD plates were sealed with cellotape (Tixo) and incubated at 37°C in a specially designed field incubator and the WHO plates were placed in candle jars and incubated at 37°C in a water bath. After 24-40 hours, a thick smear from each well was stained with 1.5% Giemsa solution (pH 7.2) and schizonts per 200 asexual parasites were enumerated. Schizont maturation for each drug concentration was expressed as percentage of control. An isolate was considered resistant if growth was observed in the well containing 5.7 pmols. of chloroquine (WHO Monograph Series, 1981).

RESULTS AND DISCUSSION

Out of 207 fever cases examined 35 were with *Plasmodium falciparum* infection of which 20 were found suitable for conducting tests. Age of selected patients ranged from 1 to 35 years and parasitaemia on D-0 ranged from 500-70,000 mm^3.

In vivo test on 20 patients showed chloroquine resistance at R-I level in 6, R-II in 5 and R-III in 2, and 5 were sensitive. The two remaining were parasite negative on D-7 but their D-14 and D-28 smears were not available for examination and hence the results were inconclusive. Of the 6 R-I type, the D-3 smears were negative in all, D-7 smears were positive in 5 and D-14 smears were positive in all the 6. The case which became positive on D-14 was considered to be due to recrudescence because the study was conducted during non-transmission season. The overall response of the 20 patients (Fig. 1) indicated a RII type of resistance. Urine samples of 19 patients examined were positive for chloroquine on D-3, confirming drug absorption. In one, urine sample was not available on D-3.

In the *in vitro* tests, the results were similar in both NICD and WHO plates. Adequate growth was observed in 7 out of 16 (43%) specimens examined. Poor success rates in micro *in vitro* tests have been reported by other workers also (Lamont and Darlow, 1982; Smrkovski *et al.*, 1983). The reasons are yet to be elucidated. Table I gives results of micro *in vitro* tests conducted in respect of 7 successful specimens in our series by the NICD and WHO methods. Schizont maturation in control wells of NICD plate was 8 to 66% with a mean 26.14% and that of WHO plate was 17 to 58% with mean 31.85%. The difference was not statistically significant

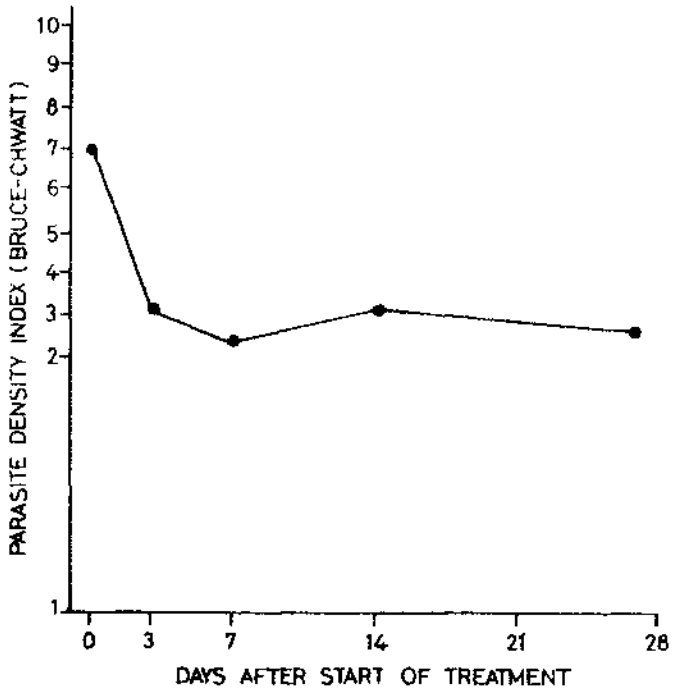


Fig. 1: WHO *in vivo* extended test on 20 patients at Sonapur PHC area (Assam).

(t-test). Responses of the 7 successful isolates to chloroquine given in Table I show that in both the plates the same 4 specimens were resistant and 3 sensitive. Inhibitory concentrations for the resistant strains in the NICD plate were 8 pmols. for one, 16 pmols. for another and 32 pmols. for the remaining two. Whereas in WHO plate, they were 8 pmols. for two and 16 pmols. and 32 pmols. for one each. However, when linear regressions were fitted separately for the responses in NICD and WHO plates (Fig. 2), the regression co-efficients were -56.2 and -60.7 respectively. The difference was not statistically significant indicating that the results in the two plates were similar. This finding further confirms the utility of the sealing technique for conducting micro *in vitro* tests already reported (Gajanana *et al.*, 1982).

Comparison of results of *in vivo* and *in vitro* tests in respect of 7 specimens showed that there was agreement in 6 (Table 1). The discrepancy in one patient (M 5) resistant *in vivo* but sensitive *in vitro*, might be due to improper absorption of the drug. The urine specimen of this patient was not available for test on D-3. It was further noted that R-II and R-III *in vivo* was associated with inhibitory concentrations of 3.2 pmol./ul of blood or more in the micro *in vitro* test. This is close to 2.5 pmols./ul of blood in the macro test observed by others (Wernsdorfer and Kouznetsov, 1980).

The present study which has clearly identified chloroquine resistant malaria of the R-II and R-III types in the study site by both *in vitro* and *in vivo* tests may now call for a revised drug

Table 1. Chloroquine sensitivity of *P. falciparum*: Comparison of micro *in vitro* and *in vivo* tests

Case	Sex/ age	*In vivo* test					Remark	*In vitro test*													
		Asexual Parasites/ mm³ blood						Schizont maturation % of control													
										Chloroquine pmol/ well											
		D 0	D 3	D 7	D 14	D 28		Control		1		2		4		5.7		8		16		32	
								a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
S1	F/17	7072	340	20	NA	NA	R-II	66	17	96	100	98	100	75	76	30	35	31	0	30	0	0	0
M9	F/1	6720	Neg.	Neg.	Neg.	Neg.	S	13	23	-	-	-	-	0	8	0	0	0	0	0	0	0	0
M5	M/25	2850	Neg*	880	4300	Pos.	R-I	22	39	109	82	80	-	63	64	0	0	0	0	0	0	0	0
D1	F/5	30000	Neg.	250	200	Pos.	R-I	8	17	-	-	-	-	100	100	75	40	100	0	0	0	0	0
T1	F/1	5644	238	170	NA	NA	R-II	52	58	86	98	73	94	57	74	61	55	28	37	9	3	0	0
T3	F/2	7430	7850	11424**	Neg.	Neg.	R-III	12	29	83	75	91	79	100	38	83	31	0	13	0	0	0	0
A1	F/4	2150	2000	Neg.	NA	NA	? S@	10	40	-	-	-	-	0	0	0	0	0	0	0	0	0	0

a = NICD Method ; b = WHO Method , - = Not tested

NA = Not available

* = No urine test : ** = Cured with Metakelfin

@ = Smears on D 14 and D 28 not available. Hence ? Sensitive

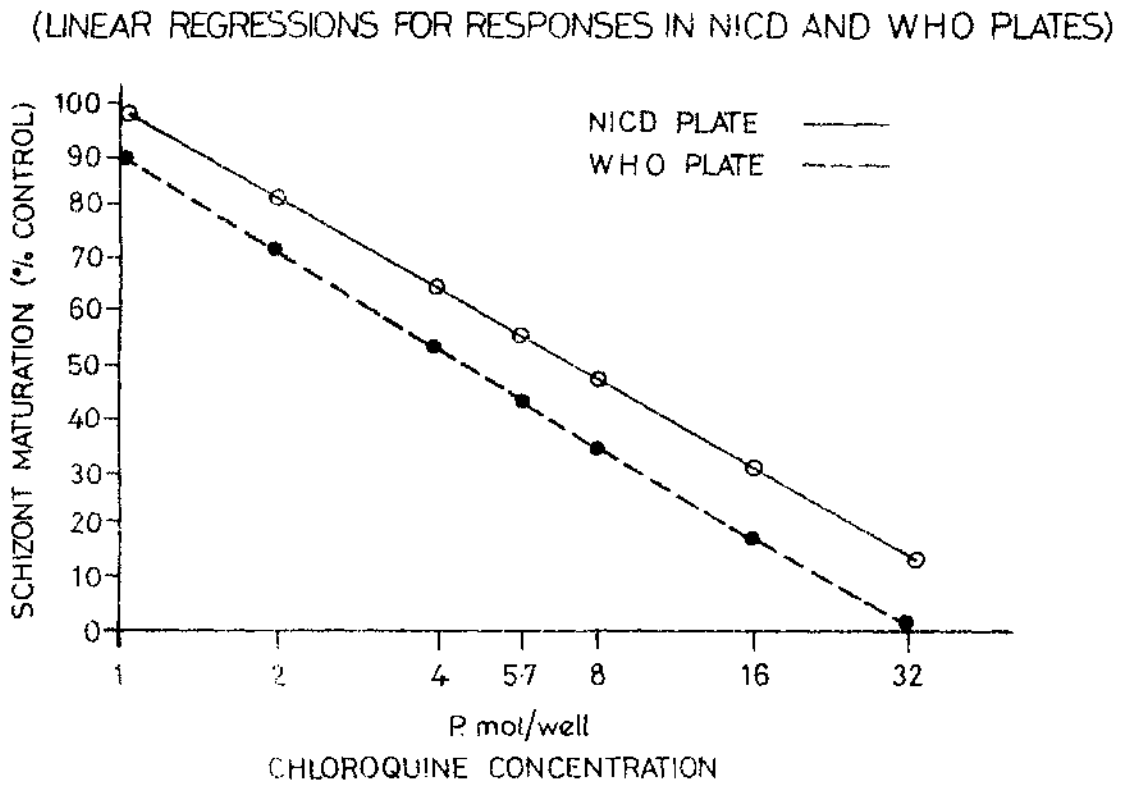


Fig. 2: Micro *in vitro* tests on 7 *Plasmodium falciparum* isolates at Sonapur PHC area (Assam).

policy for Sonapur PHC Area. Alternative antimalarial drugs are indicated after assessing clinical responses to them.

ACKNOWLEDGEMENTS

This study was supported by a grant-in-aid of the Indian Council of Medical Research, New Delhi. Authors are grateful to the Director, National Malaria Eradication Programme, for extending all facilities for carrying out this study and for critically evaluating the paper. Thanks are due to the Regional Director, Shillong and the staff of Health Services, Assam State, for their enthusiastic co-operation. Able technical assistance by the staff of ICMR Monitoring Team, Shillong and Shri J.K. Gupta is highly appreciated. The statistical analysis was carried out by Shri Ajit Mukherjee, Statistician, ICMR Scheme, National Institute of Communicable Diseases.

REFERENCES

1. Bruce-Chawtt, L.J. (1980). *Essential Malariology.* (William Heinemann Medical Books Limited, London): 145.
2. Gajanana, A., S. Sinha and A.N. Raichowdhuri (1982). Micro *in vitro* chloroquine sensitivity test for *Plasmodium falciparum* without the use of candle jar. *Indian J. Med. Res.*, **76**: 195–198.
3. Lamont, G. and Brian Darlow (1982). Comparison of *in vitro* pyrimethamine assays and *in vivo* response to sulphadoxine-pyrimethamine in *P. falciparum* from Papua, New Guinea. *Trans. R. Soc. Trop. Med. Hyg.*, **76**: 797–799.

4. Pattanayak, S., R.G. Roy, D. Phukan and B.N. Barkakaty (1979). Chloroquine resistance in *P. falciparum* in Assam State. *Indian J. Med. Res.*, **70** (Suppl.): 14–19.
5. Rieckmann, K.H., L.J. Sax, G.H. Campbell and J.E. Mrema (1978). Drug sensitivity of *P. falciparum*: An *in vitro* microtechnique. *Lancet*, **I**: 22–23.
6. Smrkovski, L., L. Stephen, L. Hoffman, Purnomo, R.P. Hussein, Sofyain Masbar and Liliana Kurniawan (1983). Chloroquine resistant *Plasmodium falciparum* on the Islands of Flores, Indonesia. *Trans. R. Soc. Trop. Med. Hyg.*, **77**: 459–462.
7. Sehgal, P.N., M.I.D. Sharma, S.Y. Sharma and S. Gogai (1973). Resistance to Chloroquine in falciparum malaria in Assam State. *J. Com. Dis.*, **5**: 175–180.
8. Weinsdorfer, W.H. and R.L. Kouznetsov (1980). Drug resistant malaria — occurrence, control and surveillance. *Bull. WHO*, **58**: 341–352.
9. *WHO Monograph Series No. 27* (1981). *Chemotherapy of Malaria*. (2nd Edition): 214–218.

Indian Journal of Malariology
Vol. 21, June 1984, pp. 31–36.

Changes in Concentration of Lymphocyte Subpopulations in Rhesus Monkey during *Plasmodium knowlesi* Infection and in Drug-cured Immune Monkeys

PAWAN SHARMA[1], MUJTABA HUSAIN[1] and G.P. DUTTA[1]

Determination of the concentrations of T and B cells by E and EAC rosetting techniques, respectively, in 71 normal rhesus monkeys revealed that T cells constituted on average 53.42±2.64% and B cells constituted 35.65±2.09% of the lymphocytes in the peripheral blood. Following infection with *Plasmodium knowlesi*, an early increase in the percentage values of both T and B cells was detected. Subsequently, concomitant with the rise in parasitaemia, a marked decline in both T and B cells was observed. Thus at parasitaemia ranging from 25 to 40%, T-cells averaged 38.13±4.92% and B-cells 26.29±2.59%. At peak parasitaemia (42 to 90%), the concentration of T-cells declined to 26.45±6.98% and of B-cells to 10.7±3.83%. Following radical cure of the infection at high parasitaemia (22.62±12.28%), monkeys rapidly regained the pre-infection levels of T, B and null cells. These levels were maintained almost unaltered even after rechallenge 10 weeks later. Hyperimmune monkeys were found to have elevated levels of T (55.48±3.21; $p<0.01$) and B cells (35.46±1.26; $p<0.01$) which were maintained despite two challenge infections with massive inocula.

INTRODUCTION

Plasmodium knowlesi infection in the rhesus monkey (*Macaca mulatta*) follows a characteristically rapid and fatal course resulting in the death of host by day 10 post-inoculation (Dutta *et al.*, 1981). Acute malaria infections usually induce a state of generalized depression of both humoral and cellular immune responses as reported in the clinical studies in humans (Greenwood *et al.*, 1972; Wyler, 1976) as well as experimental studies in the rodents (Weinbaum *et al.*, 1978). Current experimental evidence from the studies on rodent malaria suggests that the development of protective immunity against malaria is T-cell dependent (Gravely *et al.*, 1976) which gets implicated as helper cell in the establishment of a humoral response (Roberts *et al.*, 1977). Although *P. knowlesi* infection in the rhesus monkeys is known to resemble closely the course of *P. falciparum* infection in man, only limited information is available in the published literature on the changes in the lymphocyte subpopulations in the former infection (Taylor *et al.*, 1980; Chandanani *et al.*, 1981). Moreover, no information is available on the levels of T and B lymphocytes in the monkeys which have been cured at high parasitaemia or in immune monkeys which are refractory to infection. The present study deals with the determination of lymphocyte subpopulaions in the peripheral

Accepted for publication: 2 June 1984.
[1]Division of Microbiology
Central Drug Research Institute
Lucknow-226001, U.P.

blood of rhesus monkeys during acute *P. knowlesi* infection and in the immunized animals.

MATERIAL AND METHODS

This study included 77 normal healthy rhesus monkeys, weighing 4–6 kg., tuberculin negative and without any evidence of plasmodial infection. Of these, 26 were subsequently infected with *P. knowlesi* (strain W) by intravenous inoculation of 1×10^6 parasitized red cells from an infected monkey. The daily parasitaemia was monitored by examination of thin blood smears stained with Giemsa (Dutta *et al.*, 1981).

Another batch of 8 monkeys showing high parasitaemia (22.62±12.28%) was given radical curative treatment with chloroquine (20 mg. base/kg. daily) from day 9 to 11 and followed for changes in the concentrations of T and B cells in the peripheral blood. Six of these monkeys were rechallenged on day 80 with 1×10^5 parasitized erythrocytes and investigated for cellular changes.

Seven monkeys immunized by drug-cured infections (four monkeys from the present study and 3 monkeys immunized by Dutta and Singh [1981] in an earlier study) were also assayed for concentrations of T and B cells. These were then challenged thrice at intervals of 20 days, using inocula of 1×10^7 and 1×10^9 parasitized erythrocytes respectively. These monkeys were also assayed for the concentration of T and B lymphocytes in the peripheral blood.

Peripheral blood lymphocytes of monkeys were separated by density gradient centrifugation according to Boyum (1968), at different levels of parasitaemia. Their viability was checked by trypan-blue dye exclusion. Percentage of monocytes was determined by allowing them to phagocytose latex particles (Weir, 1978) and excluded from the final count. Viable cells were suspended at a concentration of 4×10^6 lymphocytes per ml of the Hank's balanced salt solution (HBSS), pH 7.2, supplemented with 10% inactivated autologous serum.

Estimation of T and B lymphocytes was carried out by enumerating E-rosette forming cells (E-RFC) and EAC-rosette forming cells (EAC-RFC) respectively, according to methods of Jondal *et al.* (1972) as modified by Malaviya *et al.* (1974).

Briefly, for estimation of T-cells, equal volumes of lymphocyte suspension (4×10^6 cells per ml) and washed sheep erythrocytes suspension (0.5%) in the HBSS supplemented with serum, were mixed together and incubated at 37°C for 15 minutes. The suspension was then centrifuged at 200×g for 5 min. Without disturbing the sedimented cells, the tubes were transferred to the ice bath and kept at 4°C overnight. Next morning the suspension was examined for rosette-forming cells after the suspension was fixed with glutaraldehyde and stained with methylene blue. Any lymphocyte with three or more sheep erythrocytes adhering to it was taken as the rosette forming cell. A minimum of 200 lymphocytes were counted from each preparation. Each sample was tested in duplicate.

For estimation of B-cells, sheep erythrocytes coated with sub-agglutinating concentration of antisheep haemolysin (Span Diagnostics, Surat) and guineapig complement were used. Equal volumes of this sheep erythrocytes suspension (0.5%) and lymphocyte suspension (4×10^6 cells per ml) were mixed together and incubated at 37°C for 1 hour. Further procedure followed was similar to that described for T-cells, except that the rosettes were resuspended by vigorous shaking prior to fixation with glutaraldehyde. Any lymphocyte with 8 or more sheep erythrocytes adhering to it was taken as the rosette forming cell.

Percentage of null cells was estimated by subtracting the sum of the percent T and B cells from 100. Results were analyzed statistically using Student's 't' test.

RESULTS

Data on six normal monkeys presented in Table 1 shows the reproducibility of the rosetting techniques as evidenced by concentrations of E-rosette-forming cells (T cells) and EAC rosette-forming cells (B cells) on four different days. These results showed that there was a slight variation in the concentration of T, B and null cells from monkey to monkey or in day to day estimations, but this variation was not significant statistically. Thus T cells in these monkeys on day 0, 5, 10 and 15 averaged 52.83±3.03, 52.14±1.88, 52.83±1.16 and 52.66±1.85% respectively; the corresponding values for B cells were 34.16±1.51, 34.05±1.12, 34.54±0.89 and 35.35±3.64% respectively, and for null cells 13.01±3.12, 13.79±2.43, 12.62±1.72 and 11.98±5.28 respectively.

Concentrations of T cells (53.42±2.64%), B cells (35.65±2.09%) and null cells (10.93±3.1%) in another 71 normal rhesus monkeys (Table 2), were comparable to those summarized in Table 1. Following infection with *P. knowlesi*, there was, initially, a slight increase in the percentage of T (55.08±3.74) and B cells (37.08±2.67) with concomitant decline in the null cells (7.83±4.49). Subsequently, however, with rise in parasitaemia there was a rapid decline in the concentrations of both T and B cells. These changes were found to be statistically significant ($p<0.001$). Thus at the peak parasitaemia (range 42 to 90%), T cells averaged 26.45±6.98 and B cells 19.70±3.83%; as a consequence, 53.84±11.17% of the lymphocytes were found to be null cells.

Results summarized in Table 3 show that following radical cure of the infection with chloroquine at high parasitaemia (22.62 ± 12.28%), the monkeys rapidly regained the preinfection levels of the T, B and null cells, these levels were maintained subsequently. Following

Table 1. Reproducibility of rosetting techniques for enumeration of T and B lymphocyte cells in peripheral blood of normal rhesus monkeys (mean ± S. D.)

Day	Parameter	Monkey No.						Mean±S.D.
		1324	1325	1326	1327	1356	1357	
0	T cell (%)	49.30	50.00	56.40	52.60	52.35	56.34	52.83 ± 3.03
	B cell (%)	35.70	34.30	34.20	35.20	31.34	34.22	34.16 ± 1.51
	Null cell (%)	15.00	15.7	9.4	12.2	16.31	9.44	13.01 ± 3.12
5	T cell (%)	50.13	50.76	55.34	51.56	51.85	53.24	52.14 ± 1.88
	B cell (%)	34.67	33.83	35.21	34.76	32.05	33.82	34.05 ± 1.12
	Null cell (%)	15.20	15.41	9.45	13.68	16.1	12.94	13.79 ± 2.43
10	T cell (%)	51.54	51.83	54.05	52.34	54.37	52.84	52.83 ± 1.16
	B cell (%)	33.85	35.58	34.54	33.89	35.71	33.71	34.54 ± 0.89
	Null cell (%)	14.61	12.59	11.41	13.77	9.92	13.45	12.62 ± 1.72
15	T cell (%)	54.14	53.51	53.13	53.88	52.18	49.15	52.66 ± 1.85
	B cell (%)	40.12	38.84	34.25	34.61	34.31	30.00	35.35 ± 3.64
	Null cell (%)	5.74	7.65	12.62	11.51	13.51	20.85	11.98 ± 5.28
Mean ± SD	T cell (%)	51.28	51.52	54.73	52.59	52.68	52.89	
		±2.12	±1.52	±1.43	±0.96	±1.14	±2.94	
	B cell (%)	36.08	35.63	34.55	34.61	33.35	32.94	
		±2.79	±2.26	±0.46	±0.54	±2.02	±1.97	
	Null cell (%)	12.64	12.84	10.72	12.79	13.96	14.17	
		±4.60	±3.73	±1.57	±1.12	±2.98	±4.79	

Table 2. Changes in the concentration of T and B Lymphocytes in the peripheral blood of rhesus monkeys infected with *Plasmodium knowlesi* (mean ± S.D.)

Parasitaemia	No. of animals	T-cell (%)	B-cell (%)	Null cells (%)
0 (Pre-inoculation)	71	53.42 ± 2.64	35.65 ± 2.09	10.93 ± 3.1
0.01 to 1%	20	55.08 ± 3.74	37.08 ± 2.67	7.83 ± 4.49
1.2 to 5%	18	48.77 ± 3.98*	35.46 ± 2.69	15.76 ± 6.03
5.1 to 20%	14	42.80 ± 3.63*	30.85 ± 2.20*	26.34 ± 5.11*
25 to 40%	7	38.13 ± 4.92*	26.29 ± 2.59*	37.57 ± 7.47*
42 to 90%	9	26.45 ± 6.98*	19.7 ± 3.83*	53.84 ± 11.17*

* $p<0.001$ as compared to the pre-inoculation values.

Table 3. Changes in peripheral T, B and null cell concentration (%) in rhesus monkeys infected with *P. knowlesi* and cured at high parasitaemia (mean ± S.D.)

Phase of infection	Day of Infection	Parasitaemia (%)	Concentration (%) of		
			T cells	B cells	Null cells
Preinfection	0	Nil	52.65	32.73	14.61
n = 8			±1.41	±2.08	±2.65
Infection	6	0.25±0.25	56.32	36.02	7.71
n = 8			±1.45	±1.23	±1.15
	9	22.62±12.28	44.50	26.62	28.86
			±5.76	±2.40	±7.61
Post treatment*	14	Nil	54.91	34.98	10.11
			±1.30	±1.65	±2.37
n = 8	21	Nil	54.27	34.13	11.59
			±0.84	±1.28	±1.61
	28	Nil	53.30	33.39	13.30
			±1.35	±2.28	±1.79
	49	Nil	52.76	34.28	12.95
			±1.46	±1.43	±1.88
	56	Nil	53.31	33.53	13.16
			±1.53	±1.73	±3.00
	70	Nil	50.86	33.73	15.41
			±2.87	±2.54	±2.42
Rechallenge**	84	3.64±3.21	51.29	30.13	18.71
n = 6			±1.76	±0.72	±2.05
n = 6	86	7.75±11.62	49.53	31.76	18.70
			±6.97	±3.85	±10.81
n = 4	91	Nil	55.29	34.72	9.99
			±0.68	±0.79	±0.20
n = 4	98	Nil	55.50	36.57	7.92
			±1.73	±1.06	±2.71

*Radical cure with chloroquine 20 mg. base/kg.×3 days from day 9-11.
**Challenge with 10^5 parasitised RBC on day 80.

rechallenge on day 80 with 1×10^5 parasitized erythrocytes, concentration of T and B cells suffered a slight depletion which was rapidly overcome as the animals controlled the infection and finally cleared the parasitaemia, without chemotherapeutic intervention. Thus chemotherapeutic intervention. Thus concentrations of T and B cells on day 98 of this respectively and these values were significantly elevated compared to the pre-infection values ($p<0.01$).

Table 4 summarises the findings about the concentration of T and B cells in the hyperimmune monkeys. As a result of the challenge with 1×10^7 parasitised erythrocytes, levels of T cells declined from the pre-challenge value of 55.48 ± 3.21% to 51.80 ± 2.19 and B cells from 35.46 ± 1.26% to 32.80 ± 3.45%. But this decline was subsequently made up and the slightly higher levels of both T and B cells were maintained despite two additional challenges with 1×10^9 parasitized erythrocytes.

Table 4. Concentration (%) of peripheral T and B lymphocytes in rhesus monkeys immunized against *P. knowlesi* (mean±S.D.)

Challenge Inoculum	Day after Challenge	Concentration (%) of	
		T cells	B cells
1×10^7	0	55.48±3.21	35.46±1.26
n = 7	6	51.80±2.19	32.80±3.45
1×10^9	0	54.76±1.11	34.74±3.52
n = 7	6	54.70±1.55	34.21±1 36
1×10^9	0	55.06±1.51	36.00±1.59
n = 7	6	55.36±1.95	35.51±1.02

Values on day 0 represent prechallenge values for respective challenge.

DISCUSSION

Our results regarding concentration of T and B cells in the peripheral blood of normal rhesus monkeys are in broad agreement with those reported by Malaviya *et al.* (1974) and Chandanani *et al.* (1981). However, slightly higher values for T cells (57.7 ± 6.99) were reported by Ganguly *et al.* (1977) while drastically low values for both T (18.87 ± 7.8) and B cells (5.8 ± 1.8) have been reported by Bazaz Malik (1981).

Results of the present study also indicate that in naive monkeys there exists an inverse correlation between the number of T and B cells and the development of acute parasitaemia. These findings confirm the similar observation made by Chandanani *et al.* (1981). However, Taylor *et al.* (1980), reported preferential depletion of T cells alone during acute *P. knowlesi* infection in the rhesus monkeys. It appears that massive depletion of T and B cells which are considered to be of prime importance for establishing effective immune response severely curtails the host's capability to withstand *P. knowlesi* infection. Although exact mechanism of the processes involved in depletion of lymphocyte subpopulations is not yet understood, the role of massive antigenemia and lymphocytotoxic antibodies has been implicated (Wells *et al.*, 1980; Chandanani *et al.*, 1981).

Our observations on the monkeys cured at high parasitaemia suggest that the depletion of lymphocytes resulting from acute malaria infection is not permanent. Chemotherapeutic measures instituted to cure acute infection give an opportunity to the host to regain the normal level of T and B cells. Recovery in the levels of these cells was remarkably rapid, the normal levels being regained within a couple of days after radical treatment. Unlike normal monkeys which succumb to infection, the hyperimmune monkeys resist challenge infections even with massive inocula of parasites. Consequently, the hyperimmune monkeys did not show depletion of T and B cells (Table 4). On the other hand the levels of T and B cells in these monkeys remained significantly elevated ($p<0.01$).

REFERENCES

Bazaz Malik, G. (1981). Peripheral lymphocyte subpopulation in malaria (*P. cynomolgi*). *J. Indian Assoc. Com. Dis.*, **3**: 53–57.

2. Boyum, A. (1968). Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.*, **21** (Suppl.): 77–89.
3. Chandanani, R.E., N.K. Ganguly, R.N. Prasad, M.S.A. Kabir and R.C. Mahajan (1981). Cellular studies in rhesus monkeys during acute, protracted and reinfection stages infected with *Plasmodium knowlesi*. *Indian J. Med. Res.*, **73** (Suppl.): 45–49.
4. Dutta, G.P. and P.P. Singh (1981). Antimalarial activity of mefloquine and chloroquine against blood induced *Plasmodium knowlesi* infection in rhesus monkeys. *Indian J. Med. Res.*, **73** (Suppl.): 23–28.
5. Dutta, G.P., P.P. Singh and P. Saibaba (1981). *Presbytis entellus* as a new host for experimental *Plasmodium knowlesi* infection. *Indian J. Med. Res.*, **73** (Suppl.): 63–66.
6. Ganguly, N.K., C. Mohan, R.P. Sapru and M. Kumar (1977). T and B cell populations in the peripheral blood of rhesus monkeys. *Int. Archs. Allergy Appl. Immunol.*, **53**: 290–292.
7. Gravely, S.M., J. Hamburger and J.P. Kreier (1976). T and B cell population changes in young and adult rats infected with *P. berghei*. *Infect. Immun.*, **14**: 178–183.
8. Greenwood, B.M., A.M. Bradley-Moore, A. Palit and A.D.M. Bryceson (1972). Immunosuppression in children with malaria. *Lancet*, **1**: 169–172.
9. Jondal, M., G. Holm and H. Wigzell (1972). Surface markers on human T and B lymphocytes. *J. Exp. Med.*, **136**: 207–215.
10. Malaviya, A.N., R. Kumar, L.N. Bhuyan and L.N. Mohapatra (1974). Rosette forming lymphocytes. A modified technique for better stability and reproducibility. Distribution in lymphoid tissue of rhesus monkeys. *Indian J. Med. Res.*, **62**: 640–647.
11. Roberts, D.W., R.G. Rank, W.P. Weidanz and T.F. Finerty (1977). Prevention of recrudescent malaria in nude mice by thymic grafting or by treatment with hyperimmune serum. *Infect. Immun.*, **16**: 821–826.
12. Taylor, D.W., S. Richmond-Crum, K.J. Kramer and W.A. Siddiqui (1980). Alterations in the distribution and proliferative responses of rhesus monkey peripheral blood and spleen cells during malaria (*P. knowlesi*) infection. *Infect. Immun.*, **28**: 502–507.
13. Weinbaum, F.I., J. Weintraub, F.K. Nkrumah, B. Charles, R.E. Evans and Y.J. Rosenberg (1978). Immunity to *Plasmodium berghei yoelii* in mice. II Specific and non-specific cellular and humoral responses during the course of infection. *J. Immunol.*, **121**: 629–636.
14. Weir, D.M. (1978). *Hand Book of Experimental Immunology*. (Blackwell Scientific Publications, Oxford).
15. Wells, R.A., K. Pavanand, S. Zolyomi, B. Permpanich and R.P. Macdermott (1980). Anti-lymphocytotoxic antibodies in sera of Thai adults infected with *Plasmodium falciparum* or *Plasmodium vivax*. *Clin. Exp. Immunol.*, **39**: 663–667.
16. Wyler, D.J. (1976). Peripheral lymphocyte subpopulations in human falciparum malaria. *Clin. Exp. Immunol.*, **23**: 471–476.

Indian Journal of Malariology
Vol. 21, June 1984, pp. 37-44.

Experimental Malarial Infection II—*Plasmodium berghei* Infection in Normal and B-cell Deficient Mice

V. PADMAVATHI[1], R. KUMAR[1], A.N. MALAVIYA[2] and L.N. MOHAPATRA[1]

Experimental malarial infection in normal swiss mice with 10^5 *P. berghei* infected RBC resulted in a fatal disease which lasted for about 4 weeks. B-cell deficient mice suffered from a more serious disease in comparison to the normal animals during the primary infection. However, after radical cure of the primary infection, the B-cell deficient animals displayed no handicap in dealing with the reinfection. The results suggested that cell-mediated immune responses alone are sufficient to deal with malarial reinfection. Furthermore, the B-cell deficient animals showed better resistance to reinfection than normal animals, suggesting that antibodies may actually be harmful in chronic malaria.

INTRODUCTION

The precise contribution by humoral antibodies and cell-mediated immune responses in protection against malarial infection remains undefined. In the preceding paper, we have shown that the T-cells are required for recovery during primary malarial infection as well as for immunity against reinfection (Padmavathi *et al.*, 1983). The T-cells are necessary not only for the establishment of cell-mediated immune responses, but also as helper cells for adequate antibody production (Jayawardena *et al.*, 1977; Weinbaum *et al.*, 1976). Further experiments were, therefore, planned to study the course of experimental malarial infection in normal and B-cell deficient mice. The present paper describes these results.

Accepted for publication: 2 June 1984
[1]Department of Microbiology
All-India Institute of Medical Sciences
New Delhi-110 029.
[2]Department of Medicine
All-India Institute of Medical Sciences
New Delhi-110 029.

MATERIAL AND METHODS

The methods for infecting the mice with *Plasmodium berghei* and follow up of the course of infection by studying PCV, mortality and parasitaemia have been described previously (Padmavathi *et al.*, 1983).

Preparation of B-cell deficient mice

Sheep anti-mouse IgM: One milligram of mouse IgM (Myeloma Protein MOPC 1104) in 2.5 ml distilled water was emulsified with an equal volume of Freund's complete adjuvant (FCA). It was given to sheep intramuscularly four times at 15 days intervals. The sheep was bled 7 days after the last injection and the serum was tested against mouse IgM in Ouchterlony. The gel diffusion titre of anti IgM was found to be 32-64.

New born mice received intraperitoneally (i.p.) 7 daily injections of 0.05 ml of sheep anti-mouse IgM starting from the day of birth. Thereafter, the treatment was continued by injecting 0.1 ml antiserum on alternate days till the end of the experiment. Control mice were treated with normal sheep serum in the same way.

Establishment of B-cell deficiency

Five mice from each group were chosen and examined for splenic B-cells, gammaglobulin concentration in the serum, humoral immune (HI) response to sheep erythrocytes (SRBC) and skin allograft rejection. The HI response to SRBC was assessed by enumerating the number of direct hemolysin producing cells in the spleen by the Jerne Plaque method and measuring the serum antibody concentration. Splenic B cells were counted by direct membrane immunofluorescence technique (Holborow, 1970) and serum gammaglobulin levels by paper electrophoresis (Straus, 1960). The cell-mediated immunity (CMI) was assessed by following skin allograft rejection pattern as described in the previous communication.

Histology

Tissues were collected and fixed in 10% formalin in physiological saline. Sections of 5 μ thickness were cut and stained routinely with hematoxylin and eosin.

RESULTS

Establishment of B-cell deficient mice

B-cell deficient mice were prepared by treatment with sheep antimouse IgM (see Material and Methods). The antiserum treated mice showed about 50% reduction in the serum gammaglobulin and a phenomenal decrease in the number of surface immunoglobulin positive cells in the spleen (Table 1). Further, in the antiserum treated mice, there were no plaque forming cells in the spleen and very low antibody in the serum (Table 1).

Table 1. Establishment of B-cell deficient mice

Group	Serum gamma-globulin (%)	Splenic B-cells (%)	Anti-SRBC PFC/ 10^6 WBC	HA titre (recip-rocal Units)
Normal Mice	19.4	48	300	160
Anti IgM treated mice	7-9.9	2	0	10

New born mice received 7 daily injections of 0.05 ml anti-IgM and later 0.1 ml on alternate days for six weeks. The serum gammaglobulin concentration and membrane immunoglobulin bearing B-cells in the spleen were measured. Normal mice served as controls. Groups of normal and antiserum treated mice were immunized with 10^8 SRBC and the splenic PFC and serum antibody responses were measured 5 days after immunization. Each figure represents a mean of ten animals.

Histology

The spleen of normal mice showed abundant germinal centre formation after immunization with SRBC. By contrast, in mice treated with the antiserum and immunized with SRBC, the germinal centres in the spleen did not appear and the B-dependent areas were depleted of lymphocytes, but with normal cellularity in the T-dependent area.

Skin Allograft

The mean survival time for skin allograft in B-cell deficient mice was 9.40 $\pm$ 0.55 days, whereas, in control animals it was 10 $\pm$ 0.71 days.

Table 2. PCV following intravenous infection with 10^5 *P. berghei* infected RBC

Days after infection	Normal mice	B-cell deficient mice
0	42.37±1.40	40.91±2.94
7	39.11±1.98 (9)	27.28±4.70 (9)
14	25.50±6.77 (6)	12.20±0.57 (5)
21	12.00 (1)	NT [b]

a. Parentheses indicate the number of animals tested.
b. Animals were not available for testing.

Table 3. Mortality following intravenous infection with 10^5 *P. berghei* infected RBC

	Days after infection	
	Normal mice (10) [a]	B-cell deficient mice (10) [a]
Mean mortality rate	15.4±5.12	12.2±3.8
50% mortality	16	13
100% mortality	23	17

a. Parentheses indicate the number of animals tested.

The above results indicate that treatment with sheep anti-mouse IgM leads to the depletion of B-cells in the spleen and abrogation of B-cell function alone. Therefore, these mice are hitherto referred as B-cell deficient.

Course of primary infection

PCV: The PCV at weekly intervals following i.v. injection of 10^5 infected RBC is shown in Table 2. Both the deficient as well as the normal control groups of animals developed progressive anaemia. However, the rate of anaemia development was faster in deficient mice than in the normal animals.

Mortality: The mortality figures for the two groups of animals following infection are shown in Table 3. The B-cell deficient animals died of *P. berghei* induced infection earlier than the normal mice.

Parasitaemia: All through the course of infection, the B-cell deficient animals maintained a much higher level of parasitaemia in comparison to the normal animals (Table 4).

Table 4. Parasitaemia following intravenous infection with 10^5 *P. berghei* infected RBC

Days after infection	Mean Parasitaemia/10^4 RBC		
	Normal mice	B-cell deficient mice	P Value
6	349.50±102.20 (10) [a]	935.44±207.79 (9)	<0.001
9	1032.25±96.66 (8)	2754.38±1078.63 (8)	<0.001
12	1339.00±345.10 (8)	2970.50±612.72 (6)	<0.001
15	1877.50±427.45 (6)	3806.00±644.40 (6)	<0.001
18	2672.00±271.53 (2)	NT [b]	
21	3844.40±241.93 (2)	NT	

a. Parentheses indicate the number of animals tested
b. Animals were not available for testing.

Course of secondary infection

In a series of further experiments, the infected normal (control) and B-cell deficient mice were cured at 10–15% level of parasitaemia with chloroquine. Ten days later, the cured animals were reinfected i.v. with 10^5 infected RBC.

Mortality: As many as 50% normal animals survived more than 4 weeks during secondary infection (Table 5), while during primary infection all animals died within this period. The mortality during the first 4 weeks after secondary infection was almost the same in normal and B-cell deficient mice (Table 5). In

Table 5. Mortality following primary and secondary infection with 10^5 *P. berghei* infected RBC

	Normal mice		B cell deficient mice	
	Primary infection (10)	Secondary infection (10)	Primary infection (10)	Secondary infection (10)
Died within 2 weeks	4*	3	5	4
Died between 2 4 weeks	6	2	5	0
Survived more than 4 weeks	—	5	—	6

Figures in parentheses indicate total number of animals tested

*Number of animals dead

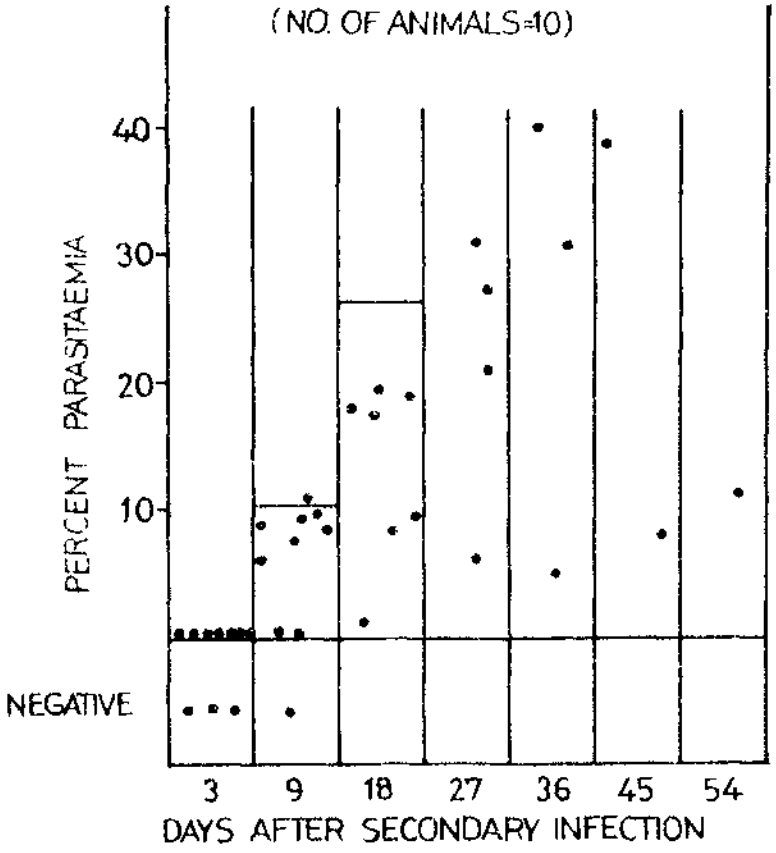


Fig. 1. Course of secondary infection in normal mice following i.v. infection with 10^5 *P. berghei* infected RBC. Horizontal bars indicate the mean parasitaemia of primary infection.

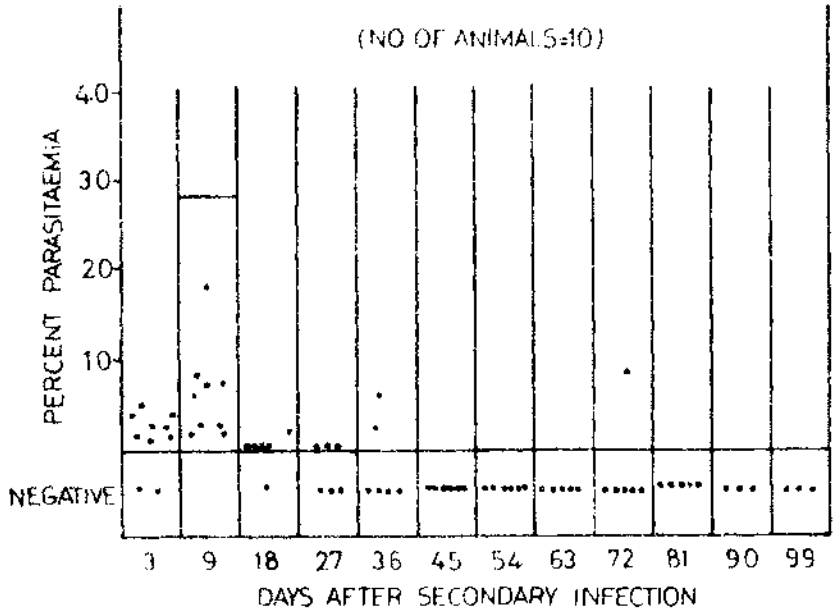


Fig. 2. Course of secondary infection in B cell deficient mice following i.v. infection with 10^5 *P. berghei* infected RBC. Horizontal bars indicate the mean parasitaemia of primary infection.

the long run, however, all normal animals died within 52-58 days after secondary infection. By contrast, in the B-cell deficient group after secondary infection, six out of ten animals survived a period of 4 weeks (3 of them survived for more than 100 days).

Parasitaemia: During secondary infection, parasitaemia in normal mice varied from animal to animal. Therefore, parasitaemia in each animal at different periods after infection is shown in Fig. 1. Although several animals died early, the parasitaemia in most of the animals was less after secondary infection than after primary infection. Similar results were obtained in B-cell deficient mice (Fig 2).

Six out of ten B-cell deficient mice survived secondary infection for more than 4 weeks. However, three of these survivors died of chronic infection (Table 6). The other three mice received i.p. 0.25 ml anti-thymocyte globulin (ATG) (Padmavathi et al., 1983) on alternate days. One of these mice developed 1-2% parasitaemia on the 106th day and died two days later. The other two mice remained negative for parasitaemia till the 125th day. It was of interest to note whether these two animals had any residual parasites. Spleen cells from the animals were transferred to normal recipients. The recipients developed no parasitaemia upto as long as 90 days after transfer. It is suggested that there were no residual parasites in the donor.

DISCUSSION

There has been a greater emphasis on the role of antibodies against experimental malarial infection (Brown, 1969; Brown and Phillips, 1974; Brown *et al.*, 1971; Diggs and Osler, 1969). The cell-mediated immune responses have received scant attention(Roberts and Weidanz, 1979; Rank and Weidanz, 1976; Spira and Silverman, 1969; Stechschulte, 1969). Our earlier studies had indicated that T-cells were required for the development of acquired immunity during primary and secondary

Table 6. Course of secondary infection in B-cell deficient mice following rechallenge with 10^6 *P. berghei* infected RBC (data on animals that survived a period of 80 days following rechallenge)

Animal number[a]	Parasitaemia on days after infection								
	3	9	18	27	36	45	54		100
1.	498[b]	810	P	P	N	N	N	Died (on day 89)	–
2.	214	218	P	N	248	N	N	Died (on day 81)	–
3.	419	614	P	P	N	N	N		N[c]
4	202	312	N	N	N	N	N		N[c]
5.	410	788	190	P	610	N	N	Died (on day 89)	–
6	189	210	P	N	N	N	N		N[c]

a B cell deficient mice received primary and secondary infections with 10^6 *P. berghei* infected RBC and the parasitaemia was followed in animals that survived more than 4 weeks.

b Values denote parasitaemia, 10^4 RBC on different days after secondary infection; N = negative parasitaemia; P = positive parasitaemia (Less than one per 10^4 RBC)

c These animals were negative for parasitaemia over 100 days and were tested for residual parasites by giving ATS. One of these mice developed low parasitaemia and died. The remaining two were used for cell transfer experiment (see the text).

malarial infections (Padmavathi *et al.*, 1983). However, it is not clear whether T-cells were required as helper cells for antibody production or for the establishment of T-effector mechanisms. In order to clearly delineate the involvement of T cells, the use of B-cell deficient animals became promising.

The results of the present study show that after primary infection, the B cell deficient animals with intact T-cell system, developed a more severe disease than the control animals. This indicates that antibody is required for immunity against blood induced primary infection. It has been shown earlier that T cell deficient mice also did not develop immunity after primary infection (Padmavathi *et al.*, 1983; Jayawardena *et al.*, 1977; Weinbaum *et al.*, 1976). From these results one may conclude that the role of T-cells in primary infection is perhaps restricted to the production of antibodies.

Our studies on the course of reinfection in normal and B cell deficient mice after radical cure of the primary infection are more informative. Indirect immunofluorescent test showed no antibody against malarial parasite in B-cell deficient mice; whereas, normal animals showed an antibody titre of 40 to 320 (reciprocal units). Firstly the B-cell deficient animals resisted the reinfection quite well. Thus, in the total absence of antibodies, the T-cells alone could generate immunity against reinfection by the blood stages of malarial parasite. Similar observations were reported in *P. yoelii* infection in mice (Roberts and Weidanz, 1979). Secondly, in the long run, the B-cell deficient animals proved to be able to resist reinfection even better than the normal animals. Several B-cell deficient animals were still surviving when all normal animals had died. Thirdly, there was no viable malarial parasite in several surviving B cell deficient mice following reinfection. These animals remained healthy even after ATG treatment and had no residual parasite in their body. Therefore, we suggest that antibodies produced in normal animals help in protecting the malarial parasite from deleterious effects of the CMI. The parasites continue to survive in such immunized normal hosts and maintain a chronic state of the disease. Further work is needed to confirm this contention.

The overall conclusion that can be drawn from the present study is that antibodies may be important to check the progress of infection in the early stages. Perhaps the CMI develops slowly in comparison to antibodies and, therefore, in early stages of primary infection it is unable to show its effect. However, the CMI plays a dominant role in immunity in the later stages of infection and antibodies may inhibit the development of CMI. In the absence of antibodies, the protective role of CMI is well pronounced. These results may be important with regard to the efforts for vaccine development, particularly the merozoite vaccine. It appears that the merozoite vaccine must induce CMI rather than antibodies, in order to be protective.

ACKNOWLEDGEMENTS

This work was supported by the Council of Scientific and Industrial Research and Indian Council of Medical Research, New Delhi, India. We sincerely thank Dr. L.H. Miller, N.I.H., U.S.A. for his generous gift of IgM myeloma protein and the National Institute of Communicable Diseases, New Delhi, India, for supplying *P. berghei*.

REFERENCES

1. Brown, I.N. (1969). Immunological aspects of malarial infection. *Adv. Immunol.*, **11**: 267–349.
2. Brown, I.N. and R.S. Phillips (1974). Immunity to *P. berghei* in rats. Passive serum transfer and role of the spleen. *Infect. Immun.*, **10**: 1213–1218.
3. Brown, K.N., R.S. Phillips and I.N. Brown (1971). The passive transfer of variant specific protection in malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **65**: 7–8.

4. Diggs, C.L. and A.G. Osler (1969) Humoral immunity in rodent malaria II. Inhibition of Parasitaemia by Serum antibody. *J. Immunol.*, **102:** 298-305.

5 Holborow, E.I. (1970). *Standardization in immunofluorescence*. (Blackwell Scientific Publication, Oxford and Edinburg).

6. Jayawardena, A.N., G.A.T. Targett R.L. Carter, E. Leuchars and A.J.S. Davies (1977). The immunological response of CBA mice to *P. yoelii* I *General characteristics. The effect of* T-cell deprivation and reconstitution with thymus grafts. *Immunology*, **32:** 849-859.

7. Padmavathi, V..R. Kumar, A.N. Malaviya and L.N. Mohapatra (1983). Experimental malarial infection I *Plasmodium berghei* infection in normal and T-cell deficient mice. *Indian J. Malariol.*, **20** (2): 115-123

8. Rank, R.G. and W.D Weidanz (1976). Non sterilizing immunity in avian malaria. An antibody independent phenomenon. *Proc. Soc. Exp. Biol. Med.*, **151:** 257-259.

9. Roberts, D.W. and W.P. Weidanz (1979). T-cell immunity to malaria in B-cell deficient mouse. *Am. J. Trop. Med. Hyg.*, **28:** 1-3.

10. Spira, D.T. and P.H. Silverman (1969). Antilymphocyte serum effects on *P. berghei* infection in rats. *Am. Soc. Trop. Med. Hyg., Annual Meeting, Abstract No. 179.*

11. Stechschulte, D.J. (1969). Cell mediated immunity in rats infected with *P. berghei. Milit. Med.*, **134** (Suppl.): 1147-1152.

12. Straus, R. (1960). Method for the fractionation of Lipoproteins in serum by paper electrophoresis. In *Lipids and Steroid Hormones in Clinical Medicine.* Ed. F. William Sunderman and F.W. Sunderman Jr., (J.B. Lippincott Company, Philadelphia, Montreal).

13. Weinbaum, F.I., C.B. Evans and R.E. Tigelaar (1976). Immunity to *P. yoelii* in mice. I The course of infection in T-cell and B-cell deficient mice. *J. Immunol.*, **117:** 1999-2005.

Indian Journal of Malariology
Vol. 21, June 1984, pp. 45–51.

Experimental Malarial Infection III—Protective Role of Antibodies in *Plasmodium berghei* Infection in Mice

V. PADMAVATHI[1], R. KUMAR[1], A.N. MALAVIYA[2] and L.N. MOHAPATRA[1]

The effect of adoptive transfer of specific antibody on the course of experimental malarial infection in normal, T cell deficient and B-cell deficient mice was studied. In normal and B-cell deficient mice, the administration of immune mouse serum slightly delayed the onset of parasitaemia; but once it appeared, its further progress was comparable to parasitaemia in normal animals given normal mouse serum (controls). However, in T-cell deficient mice, the administration of immune serum made no difference to the course of infection in comparison to the controls. It is concluded that immune mouse serum may be protective only when the T-cell system is intact.

INTRODUCTION

The pattern of acquired immunity to the erythrocytic stage of malaria varies widely in different host-parasite species. Observations in humans (Cohen *et al.* 1961), monkeys (Coggeshall and Kumm, 1937; Brown *et al.*, 1971) and rodents (Diggs and Osler, 1969; Brown and Phillips, 1974; Briggs *et al.*, 1970; Bruce Chwatt and Gibson, 1955) have indicated that passive transfer of immune serum can cause a fall in the existing parasitaemia or prevent parasitaemia from rising after challenge with infected erythrocytes. These studies were carried out using immunocompetent animals. By contrast, our recent studies have shown that B-cell deficient animals can withstand the blood induced malarial reinfection better than the normal intact animals (Padmavathi *et al.*, 1984). Therefore, we were interested to investigate in more details the role of antibody in protection. In the present paper, the effect of administration of immune mouse serum on experimental *P. berghei* infection in normal, T-cell deficient and B-cell deficient mice was studied.

MATERIAL AND METHODS

The details about the experimental animals used, the maintenance of *P. berghei*, preparation of T-cell deficient and B-cell deficient mice, the infection of animals, and the methods used to follow the course of infection, have been previously described (Padmavathi *et al.*, 1983 Padmavathi *et al.*, 1984).

Preparation of immune mouse serum (IMS): The IMS was prepared following the method of Poels *et al.* (1977). Normal adult mice were infected intravenously (i.v.) with 10^5 *P. berghei*

Accepted for publication: 2 June 1984.
[1]Department of Microbiology
All India Institute of Medical Sciences
New Delhi-110029
[2]Department of Medicine
All India Institute of Medical Sciences
New Delhi-110029

infected RBC. The course of infection was followed by determining parasitaemia every alternate day. At 10-15% level of parasitaemia, the animals were cured with chloroquine (320 mg/litre in drinking water given continuously for 7 days). Ten days later, the cured animals were repeatedly reinfected and cured five times. At this stage, all the animals showed a very high degree of immunity against a rechallenge infection. Ten days after the last curative regimen, all the animals were bled and their sera pooled. The pooled sera was filter sterilized and stored in aliquots at -70°C without any preservative.

The titre of the pooled immune serum was determined by indirect immunofluorescence technique using *P. berghei* infected smears and fluorescein isothiocyanate conjugated anti mouse gammaglobulin antiserum (Voller, 1964). The titre was found to be 1: 1000. The protective potential of IMS was assessed by injecting into normal and deficient mice.

RESULTS

The effect of immune serum on the course of infection in normal and T-cell deficient mice: Groups of normal and T-cell deficient mice (age and sex matched) received i.p. 0.25 ml IMS or NMS (control). One day later, they were challenged i.v. with 10^5 *P. berghei* infected RBC. The treatment with IMS/NMS was continued in the respective groups by twice a week injections till the end of the experiment. The course of infection was followed by studying packed cell

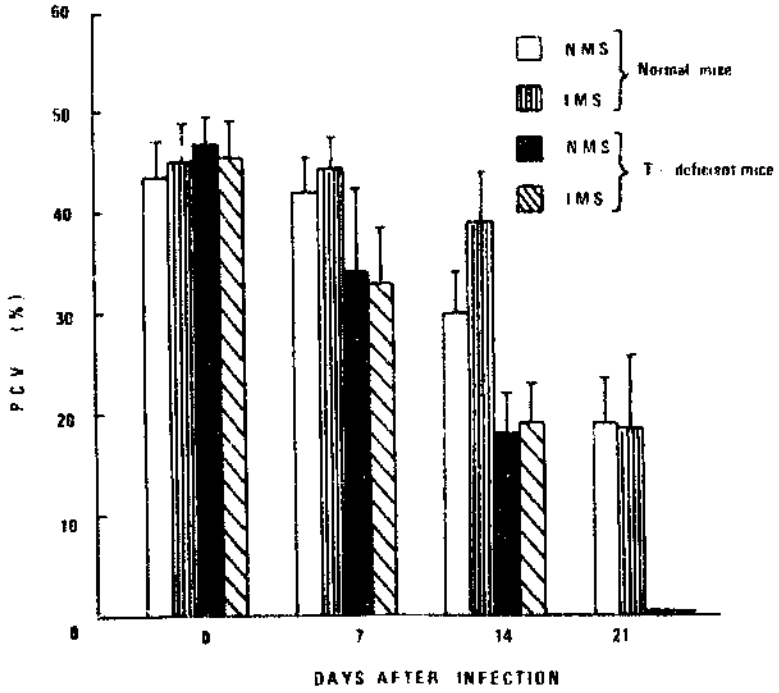


Fig. 1 Effect of immune serum on PCV in T-cell deficient mice. Normal and T cell deficient mice were treated with IMS or NMS (see material and methods). They were challenged i.v. with 10^5 *P. berghei* infected RBC and PCV was measured on different days after challenge. Values represent the arithmetic mean ± standard deviation. Zero values denote no animal was alive on that day.

volume (PCV), mortality and parasitaemia on different days after infection

PCV: The development of anaemia was slightly delayed in normal mice receiving IMS in comparison with normal mice given NMS. In T-cell deficient mice, the development of anaemia was faster than the normal intact mice.

However, the administration of IMS did not make any difference (Fig. 1).

Mortality: The infected T-cell deficient mice died earlier than the infected normal mice. The administration of IMS made no difference in mortality within the normal or T-cell deficient groups (Table 1).

Parasitaemia: The parasitaemia on different days following infection with *P. berghei* in different groups of mice is shown in Fig. 2. Administration of IMS to normal mice delayed the onset of parasitaemia by about 3 days; but once it appeared, the rate of increase was comparable to that in normal animals given NMS. The difference in parasitaemia in the initial phases of the infection was statistically significant. The T-cell deficient animals had a higher level of parasitaemia in comparison to normal animals all through the course of the infection. Moreover, within the T-cell deficient group of animals, the administration of IMS made no difference in the course of infection (Fig. 2).

The effect of immune serum on the course of infection in normal and B-cell deficient mice: The experimental protocol of infecting the animals with *P. berghei*, administration of IMS/NMS and follow up of the infected animals was the same as described above for T-cell deficient animals.

The results in normal animals with or without IMS were more or less similar to what was previously observed and described above. Therefore, the following description is restricted to the results obtained in the B-cell deficient animals only, even though the results in normal animals are shown in the tables and figures for comparison.

PCV: In B-cell deficient animals given IMS, there was only a slight delay in the development of anaemia (Fig. 3).

Mortality: The B-cell deficient animals died of infection earlier than the normal animals (Table 2). Within the B-cell deficent group of animals, administration of IMS slightly delayed the mortality (Table 2).

Table 1. Effect of immune serum on mortality in T-cell deficient mice following *P. berghei* infection.

	Mortality in days after infection in			
	Normal mice treated with		T-cell deficient mice treated with	
	NMS (10)	IMS (10)	NMS (10)	IMS (10)
Mean mortality	20.20±4.75	20.90±5.83	12.90±1.79	12.40±2.41
50% Mortality	22	22	12	13
100% Mortality	25	26	17	16

Figures in Parentheses indicate the number of animals tested

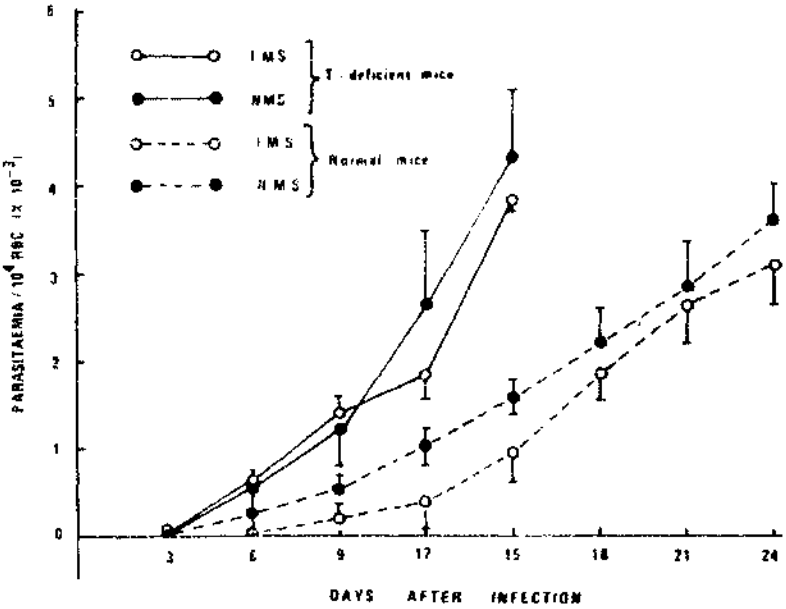


Fig. 2: Effect of immune serum on Parasitaemia in T-cell deficient mice: Normal and T-cell deficient mice were treated with IMS or NMS (see material and methods). They were challenged i.v. with 10^5 *P. berghei* infected RBC and parasitaemia was measured on different days after challenge. Values represent the arithmetic mean ± standard deviation. Zero values denote no animal was alive on that day.

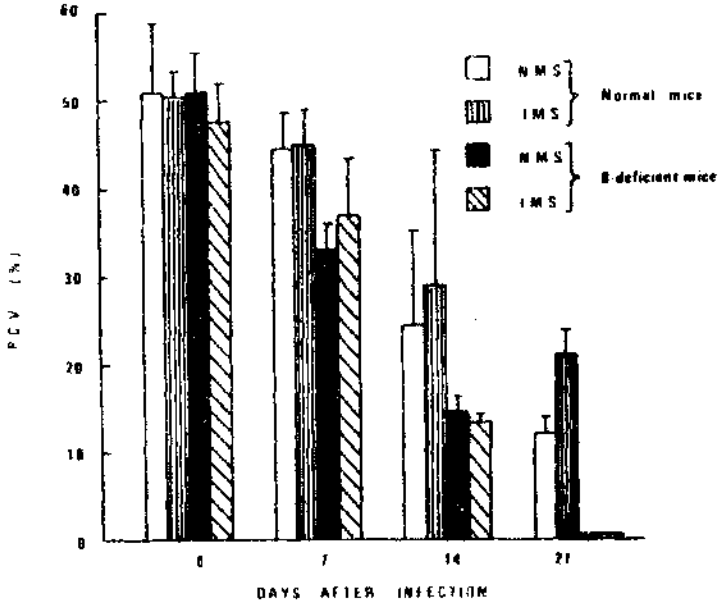


Fig. 3: Effect of immune serum on PCV in B cell deficient mice. Normal and B-cell deficient mice were treated with IMS or NMS (see material and methods). They were challenged i.v. with 10^5 *P. berghei* infected RBC and PCV measured on different days after challenge. Values represent the arithmetic mean ± standard deviation. Zero values denote no animal was alive on that day.

Table 2. Effect of immune serum on mortality in B-cell deficient mice following *P. berghei* infection

	Mortality in days after infection in			
	Normal mice treated with		B-cell deficient mice treated with	
	NMS (10)	IMS (10)	NMS (10)	IMS (10)
Mean Mortality	17.8±3.8	19.0±7.0	11.3±4.5	11.9±4.5
50% Mortality	18	23	8	12
100% Mortality	22	25	16	18

Figures in Parentheses indicate the number of animals tested.

Parasitaemia: In the B cell deficient animals, the onset of parasitaemia was earlier and the level of parasitaemia was higher in comparison to normal animals (Fig. 4). Within the B cell deficient animals, administration of IMS considerably delayed the onset of parasitaemia and the level of parasitaemia was lower throughout the course of infection in comparison to deficient animals given NMS (Fig. 4).

DISCUSSION

The role of antibody in protection against malarial infection remains controversial. However, passive transfer of immune serum has

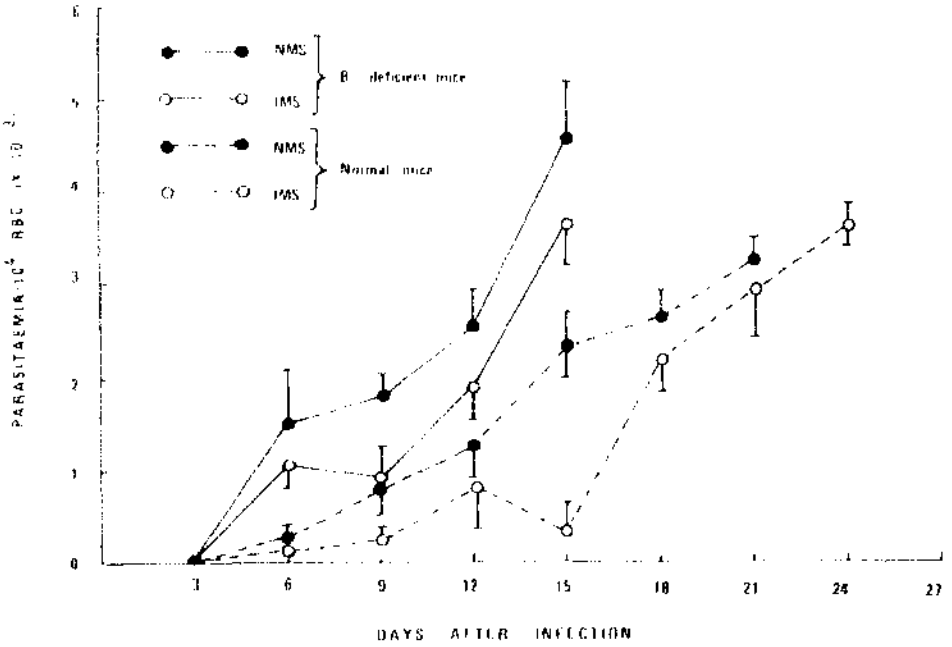


Fig. 4. Effect of immune serum on parasitaemia in B cell deficient mice. Normal and B cell deficient mice were treated with IMS or NMS (see material and methods). They were challenged i.v. with 10^7 *P. berghei* infected RBC and parasitaemia was measured on different days after challenge. Values represent the arithmetic mean ± standard deviation. Zero values denote no animal was alive on that day.

been shown to offer a certain level of protection against plasmodial infection in rats (Bruce Chwatt and Gibson, 1955; Fabiani and Fulchiron, 1953). Administration of hyperimmune-serum, reduced parasitaemia in *P. falciparum*-infected children (Cohen *et al.*, 1961).

In the present study, there was a delay in development of anaemia (Fig. 1 & 3) and parasitaemia (Fig. 2 & 4) when normal animals were given hyperimmune serum. However, the effect of hyperimmune serum was seen only in the beginning of the infection; later on the parasitaemia had gone up despite the continued administration of hyperimmune serum. Also the administration of hyperimmune serum had only a slight effect on mortality in normal animals (Tables 1 & 2). In the B cell deficient animals, the primary infection was more serious compared to normal animals (Padmavathi *et al.*, 1983; Weinbaum *et al.*, 1976). The administration of hyperimmune serum reconstituted the deficiency to a significant extent, as expected (Fig. 3, Table 2 and Fig. 4). In T-cell deficient animals also the primary infection was more severe than normal animals (Padmavathi *et al.*, 1984; Weinbaum *et al.*, 1976; Jayawardena *et al.*, 1977; Jayawardena *et al.*, 1978; Jayawardena *et al.*, 1979). However, the administration of the hyperimmune serum made no difference in the course of infection, Fig. 1., Table 1 and Fig. 2).

Recently, it has been reported that adminstration of hyperimmune serum protected intact mice completely, from a normally resolving *P. yoelii* infection, but caused only a 5–6 days delay in the onset of infection in T-cell deprived mice given hyperimmune serum (Jayawardena *et al.*, 1978). It was proposed that hyperimmune serum given to normal animals exerted an inhibitory effect, providing the intact host with a sufficient interval of time during which it could mount an active protective response against the parasite. It seems that *P. yoelii* induces massive T-cell activation leading to protective immunity (Jayawardena *et al.*, 1975). In contrast, this T cell activity elicited during the fatal *P. berghei* infection was proportionately much less than observed in a resolving *P. yoelii* infection. This lack of T-cell activity explains the inability of IMS to provide substantial immunity in normal mice.

Although the precise functional significance of T-cell mitosis observed in these infections (Jayawardena *et al.*, 1975) is uncertain, but such mitosis could be indicative of the development of cytotoxic, helper or some other T-cell-dependent effector activity such as lymphokine lymphotoxin production etc.

From these studies, one may conclude that (1) antibodies protect by delaying early parasitaemia; (2) antibodies act only in the presence of functionally intact T-cell system and (3) antibodies are unable to provide absolute protection because finally all the infected animals developed lethal disease.

So far no mechanism has been postulated where antibodies provide help to T-cells in their effector function. It would appear that cell mediated immune responses are dominating in providing immunity. This calls for further work on cell-mediated effector mechanisms, particularly with lethal malarial infections.

ACKNOWLEDGEMENTS

This work was supported by the Council of Scientific and Industrial Research and Indian Council of Medical Research, New Delhi, India.

REFERENCES

1. Briggs, N.T., B.T. Wellde and E.H. Sadun (1970). Treatment of malarial infections with homologous and heterologous antisera. *J. Parasitol.*, **56**: 35.
2. Brown, I.N. and R.S. Phillips (1974). Immunity to *P. berghei* in rats: Passive serum transfer and role of the spleen. *Infect. Immun.*, **10**: 1213–1218.

3. Brown, K.N., R.S. Phillips and I.N. Brown (1971). The passive transfer of variant specific protection in Malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **65:** 7 8.

4. Bruce Chwatt, L.J. and F.D. Gibson (1955). Transplacental passage of *P. berghei* and passive transfer of immunity in rats and mice. *Trans. R. Soc. Trop Med. Hyg.*, **50:** 47-53.

5. Coggeshall, L.T and H.W. Kumm (1937). Demonstration of passive immunity in experimental monkey malaria. *J. Exp. Med.*, **66:** 177-190.

6. Cohen, S., I.A. McGregor and S. Carrington (1961) Gammaglobulin and acquired immunity to human malaria. *Nature:* **192:** 733 737.

7. Diggs, C.L. and A.G. Osler (1969) Humoral immunity in rodent malaria. II Inhibition of parasitaemia by serum antibody. *J. Immunol.*, **102:** 298-305

8. Fabiani, G. and G. Fulchiron (1953). Demonstration *in vivo* de l'existance d'va pouvoir protecteur dans le serum des rats gueris de paludisme experimental. *Compte Rendue de la Societe de Biologie*, **147:** 99-103.

9. Jayawardena, A.N., C.A. Janeway Jr. and J.D. Kump (1979). Experimental Malaria in CBA/N mouse. *J. Immunol.*, **123:** 2532-2539.

10. Jayawardena, A.N., G.A.T. Targett, E. Leuchars and A.J.S. Davies (1978). The immunological response of CBA mice to *P. yoelli:* The passive transfer of immunity with serum and cells. *Immunology*, **34:** 157-165.

11. Jayawardena, A.N., G.A.T. Targett, E. Leuchars, R.L. Carter, M.J. Doenhoff and A.J.S Davies (1975). T-cell activation in murine malaria. ***Nature*, 258:** 149-151.

12. Jayawardena, A.N., G.A.T. Targett, R.L. Carter, Elizabeth Leuchars and A.J.S. Davies (1977). The immunological response of CBA mice to *P. yoelli* I: General Characteristics. The effects of T-cell. deprivation and reconstitution with thymus grafts. *Immunology*, **32:** 849-859.

13. Padmavathi, V., R. Kumar, A.N. Malaviya and L.N. Mohapatra (1983). Experimental Malarial Infection I *Plasmodium berghei* Infection in Normal and T cell Deficient Mice. *Indian J. Malariol.*, **20** (2): 115-123.

14. Padmavathi, V., R. Kumar, A.N. Malaviya and L.N. Mohapatra (1984). Experimental Malarial Infection II *Plasmodium berghei* Infection in Normal and B cell Deficient Mice. *Indian J. Malariol.*, **21** (1): 37-44.

15. Poels, L.G., C.C. VanNiekerk, M.A.M. Franken and E.H. Van Elven (1977). *P. berghei:* Formation of Secondary immune complexes in Hyperimmune mice. *Exp. Parasitol.*, **43:** 255-267.

16. Voller, A. (1964). Fluorescent antibody methods and their use in malaria research. *Bull. WHO*, **30:** 343-354.

17. Weinbaum, F.I., C.B. Evans and R.E. Tigelaar (1976). Immunity to *P. yoelli* in mice. I. The course of infection in T cell and B cell deficient mice. *J. Immunol.*, **117:** 1999 2005.

SHORT NOTE

Indian Journal of Malariology
Vol. 21, June 1984, pp. 55-56.

Chloroquine Resistant *P. falciparum* Malaria in Assam and Meghalaya

B.N. BARKAKATY[1], P.C. KALITA[1], SILPI DAS[1] and A.C. TALUKDAR[1]

Investigation on chloroquine resistance in *P. falciparum* was undertaken in Sonapur PHC of Kamrup district and Galeki PHC of Sibsagar district of Assam during October to December, 1980. It was also conducted in Baghmara PHC of west Garo Hills district of Meghalaya during November, 1979 to January, 1980. *In vivo* test for chloroquine resistance was carried out on persons selected after mass blood survey conducted in the above three places. Test procedures and interpretation of data were as per the recommendation of WHO. Urine was examined for the presence of chloroquine by the Dill and Glazko method. (Lelijveld and Kortmann, 1970). A seven-day field test was carried out in Kamrup district of Assam, whereas in the other two places the field tests were extended to 28 days.

The results of the tests are given in Table 1. From the table it can be seen that 8 cases out of 23 examined, showed R_1 resistance to chloroquine in Sonapur PHC. In Galeki PHC, 16 out of 18 and in Baghmara PHC, 5 out of 14 showed R_1 resistance to chloroquine.

In the Khasi Hills district of Meghalaya, which is adjacent to Kamrup district of Assam, R_2 resistance was detected in 1975 (Chakraborty *et al.*, 1979). Galeki PHC study area was adjacent to Nagaland where chloroquine resistance was detected earlier. Baghmara PHC is bordering Dalu PHC of Garo Hills district of Meghalaya, where chloroquine resistance has already been reported (Chakraborty *et al.*, 1979).

Thus, it can be seen that the area under chloroquine resistant *P. falciparum* is gradually increasing. Luckily, till now most of the resistance is of R_1 grade in both Assam and Meghalaya

Accepted for publication: 12 March 1984
[1]Regional Office for Health and Family Welfare
Felli-Ville, Lumshopoh
Shillong-793014.

ACKNOWLEDGEMENTS

The authors are grateful to the Director of Health Services of Assam and of Meghalaya and their officers for the help rendered. Thanks are due to the technicians of ICMR schemes in the Regional Office for Health and Family Welfare, Shillong, for their technical help. The work was

Table 1. Results of *in vivo* tests with Chloroquine in Assam and Meghalaya

Locality	No. of cases	No. positive for asexual stages of *P. falciparum*							
		Day '0'	Day '2'	Day '4'	Day '7'	Day '10'	Day '14'	Day '21'	Day '28'
*Sonapur PHC Kamrup Dist. (Assam)	23	23/23	11/23	2/23	8/23	ND	ND	ND	ND
Galeki PHC Sibsagar District (Assam)	18	18/18	14/18	5/18	10/18	3/8	2/5	0/3	1/3
Baghmara PHC West Garo Hills District (Meghalaya)	14	14/14	12/14	4/14	2/14	0/12	0/11	ND	3/12

* 7 day Field test Numerator -- No. positive
Denominator -- No. examined
ND = Not done

carried out with the financial support of the Indian Council of Medical Research, New Delhi.

REFERENCES

1. Chakraborty, S.C., S.R. Dwivedi, Silpi Das, D. Phukan, R.G. Roy and S. Pattanayak (1979). Response of *Plasmodium falciparum* to chloroquine in Meghalaya State. *Indian J. Med. Res.*, **70:** (Suppl.): 34-39)
2. Lelijveld, J. and H. Kortmann (1970). The eosin colour test of Dill and Glazko: a simple Field Test to detect chloroquine in the urine. *Bull. WHO*, **42:** 477-479

BOOK REVIEW

The Anophelines of India (Revised edition) 1984 by T. Ramachandra Rao

C.P. Pant[1]

This is a scholarly and monumental work full of information on the subject based on comprehensive review of over 1000 references and the author's entire life experiences on the study of the mosquitoes, disease transmission and control. There are very few specialists like Dr. Ramachandra Rao who have devoted their entire lifetime to these studies. Starting from some very interesting information about the origins of the name Anopheles through ancient Greek and Sanskrit, the author follows up the work on the Anophelines of India since the publication of "Anophelini" in the Fauna of British India series by Sir Rickard Christophers. Dr. Rao has dealt with, in a masterly fashion, several subjects of current importance. In a well-balanced presentation, he describes the biology of Anophelines, their distribution, concepts of speciation and genetics, role as vectors of human and animal diseases, and introduction to mathematical epidemiology. This is followed by the principles of malaria control and the history of the campaign against malaria in India, problems which were faced, resistance to insecticides and the need for a balanced and integrated approach towards the control of malaria vectors. Information has been given on the Anophelines of neighbouring countries. In Part II, the author gives a detailed review of 51 individual species which are not only of great value to the student but also to the specialist and the person interested in the control of the species. The important species *Anopheles culicifacies*, occupies more than 50 pages of this chapter and is indeed very comprehensive.

The up-dated key to the Indian Anophelines will no doubt be of great value to the field workers. As Professor Ramalingaswamy has pointed out in his foreword, this book should be instrumental in encouraging many a future student of zoology to undertake research on Anophelines, understand better the Anophelines in general and malaria vectors in view of the problems being faced in the control of malaria globally, the appearance of this book is most timely.

Some typing errors remain in the text and there could have been more illustrations, figures and maps, etc., but these hardly detract from the usefulness of the book. At the reasonable price listed, this book should find a place in the personal library of those concerned with the Anophelines of India and, of course, every major reference library on medical entomology.

The Indian Council of Medical Research deserves praise in encouraging and bringing out this publication.

[1]Chief, Ecology and Control of Vectors
Division of Vector Biology and Control
WHO, 1211 Geneva
27-Switzerland.

INDIAN JOURNAL OF MALARIOLOGY

Notes for Contributors

1. The Indian Journal of Malariology is devoted to the publication of original research which contributes significantly to the knowledge in any field of malariology. Papers of routine and repetitive nature dealing with gross observations may not be included.

2. Manuscripts should be sent in triplicate to the Editor, Indian Journal of Malariology, Malaria Research Centre, 22, Sham Nath Marg, Delhi-110 054. Manuscript once sent to this journal implies that it is not under consideration for publication in any other journal.

3. Articles will be published at the editor's discretion in the order accepted. Date of acceptance will be that date on which copy is accepted in final form for publication.

4. The manuscript should be typewritten in English in double space on one side of the paper. Pages should be numbered consecutively. The matter should be arranged in the following order: Title, name(s) of the author(s) with address of the Institute/University (as foot notes and numbered serially in superscript), abstract, introduction, material and methods, results, discussion, acknowledgement, and references. Abstract, tables and captions of the figures should be typed on a separate sheet of paper. Line drawings should be clear and lettered in Indian ink. In case of photographs, good quality pictures should be provided. Both line drawings and photographs should be sent in duplicate, duly supplied with legends and separated from the text but allocated to sections of the text.

5. The International system of units (SI Units) should be used for all measurements. (Kindly see WHO publication, *The SI for the Health Professional*, WHO, Geneva,1977).

6. Authors should provide a short title to be used as running title, of not more than 5-6 words in case the title of the paper is long.

7. References in the text may be cited as Sharma *et al.* (1976) or (Mount *et al* , 1972; Coombes *et al.*, 1973; Mount *et al.*,1974). For references to a paper accepted for publication, the word 'in press' should appear after the title of the periodical. References to unpublished work should not be included in the list, they should be incorporated in the text itself (e.g., R.G. Roy, unpublished data or S. Pattanayak, personal communication). If the reference is to an article published without any authorship in a periodical, in place of author's name the term "Anonymous" should be used.

Titles of Periodicals cited in the references are to be abbreviated as in the *World List of Scientific Periodicals*, 4th edition, 1964.

Reference to the literature, arranged alphabetically, and numbered consecutively, must be placed at the end of the paper. The following procedure may be followed:

Research Papers

Sharma, V.P. (1976). Elimination of aziridine residues from chemosterilised mosquitoes. *Nature*, **261** (5556): 135.

Ansari, M.A., V.P. Sharma and R.K. Razdan (1978). Mass rearing procedures for *Anopheles stephensi* Liston, *J. Com. Dis.*, **10**(2): 131-135.

Books/Monographs

Rao, T.R. (1981). *Anophelines of India* (W.Q. Judge Press, Bangalore).

Landau, I. and Y. Boulard (1978). In *Rodent Malaria*, edited by R. Killick-Kendric and W. Peters (Academic Press Inc., London): 53-84.

Paper presented at Symposium/Conference

Subbarao, S.K. *Cytoplasmic incompatibility in mosquitoes*. Paper presented at the International symposium on recent developments in the genetics of insect disease vectors, Bellagio, Italy, 20-24 April, 1981.

8. Short notes should be prepared in a manner similar to the research papers and will have title, name(s) of author(s) with address of Institute/University as footnotes, acknowledgement and references.

9. Reviews would be published of the invited papers only. Book reviews may be published at the end of the journal.

10. Galley proofs of the articles will be sent to the authors for correction. Corrected proofs must be returned promptly to the editor or else the article will not be printed in the current issue or may be printed as it stands.

11. Reprints will be mailed to the senior author. Twentyfive reprints will be supplied free of charge to single author and thirty for joint authors to be distributed on an equal basis. Additional reprints may be ordered on payment when the proofs are returned. A reprint order form will be supplied with the proofs.

Note: **All correspondence regarding contribution and subscription should be addressed to the Editor, Indian Journal of Malariology, 22, Sham Nath Marg, Delhi-110 054.**

ANNOUNCEMENT

Electronmicroscope Facility at the All India Institute of Medical Sciences, New Delhi

Scientific research requires highly sophisticated and expensive equipment. It is a fact that such equipment, when acquired, very often is not put to maximum utilization due to various constraints. In order to utilize the resources/and equipments maximally, the Department of Science and Technology has initiated a programme by setting up "Regional Sophisticated Instrumentation Centres" (RSIC) in the country.

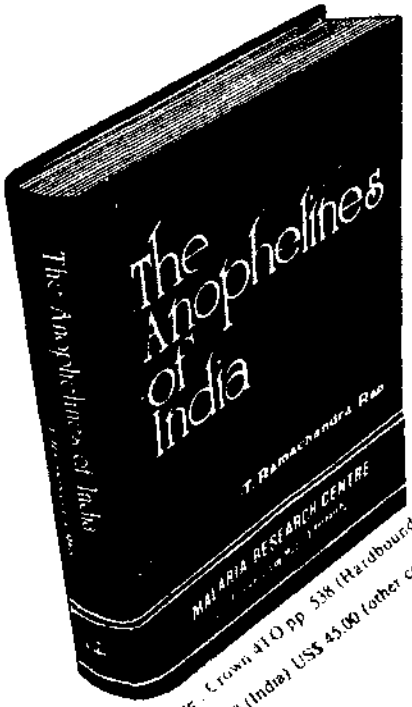
The RSIC's facilities that have been established and which are operational are located at Bombay, Calcutta, Madras, Lucknow, Bangalore, New Delhi, Nagpur, Chandigarh and Shillong. A wide range of sophisticated instruments is now available for use at the various RSICs.

This announcement pertains to the *Electronmicroscope Facility* that is operational at the *All India Institute of Medical Sciences (AIIMS), New Delhi, under the RSIC programme of Department of Science and Technology.* The EM Facility at the AIIMS will cater for scientists and investigators of all disciplines from educational and research institutions, R & D laboratories and industries on nominal charges. The facilities include a Scanning Electronmicroscope (Philips EM 501), Specimen Coating Unit and a Transmission Electron-microscope (Philips EM 300). The scientists are requested to make full use of the opportunities now available at the AIIMS for ultrastructural studies.

Besides offering research facilities, a training programme in the use and application of electronmicroscopy is being organized bi-annually.

For further details, kindly write to:

Dr. G.F.X. David
Regional Electronmicroscope Facility
Room No. 1012, Department of Anatomy
All India Institute of Medical Sciences
New Delhi 110 029, India
Tel : 665995/661831



Size : Crown 4to pp. 538 (Hardbound)
Rs 150.00 (India) US$ 45.00 (other countries)

Announcing

the Release of

THE ANOPHELINES OF INDIA

Revised Edition, March, 1984.

by

T. Ramachandra Rao

AN AUTHORITATIVE AND COMPREHENSIVE PUBLICATION WHICH BRINGS TOGETHER ALL THAT IS KNOWN ABOUT DISTRIBUTION, BIONOMICS, ECOLOGY, RELATION TO DISEASE AND BASIC PRINCIPLES OF CONTROL OF ANOPHELINE MOSQUITOES.

The monograph can be obtained by sending M.O., bank draft/postal order/cheque in favour of the Editor, Indian Journal of Malariology, Malaria Research Centre (ICMR), 22, Sham Nath Marg, Delhi-110 054, INDIA.