ABSTRACTS OF RECENT CHINESE PUBLICATIONS ON MALARIA1 (XXII)


It was previously shown that infection with Plasmodium vivax gametocytes in mosquitoes occurred only in a definite period and it was suggested that the noninfective period coincided with the time when asexual forms ruptured.

The present study is concerned with the influence of temperature on the capacity of gametocytes to develop in the mosquito gut. The results obtained from two cases with sporozoite-induced vivax malaria by means of membrane feeding at 2- to 4-hour intervals for 48 and 96 hours demonstrated that the relatively high mosquito infection rates of 61.2%, 68.2% and 63.9% corresponded to temperature peaks of 40.3°C, 40.9°C and 41.0°C respectively. Clearly, hyperpyrexia has no significant adverse effect upon the periodical infectivity of gametocytes for mosquitoes.


The present study conducted in the north-western part of Hunan Province during the period 1985-1986 is concerned with the infectivity for Anopheles sinensis of gametocytes from five sporozoite-induced infections with Plasmodium vivax. The results obtained by the membrane feeding technique demonstrated that: (1) when mosquitos were fed every 8 hours for 6 days, a remarkable 48-hour cycle of gametocyte infectivity could be observed, such regular infection cycles of alternate days representing the maturation of successive waves of gametocytes produced during the synchronous 48-hour cycle of asexual growth; (2) judging from the space between these upward and downward curves, the life span of P. vivax gametocytes might be as short as one generation of schizonts in the peripheral blood, but it is puzzling that the gametocytes show 24-hour instead of 48-hour periodic changes in infection which undergoes an asynchronous schizogonous cycle; (3) the periodical viability of gametocytes was encountered only in the very early and middle stages of illness and disappeared afterwards; (4) in two cases when mosquitos were fed every 4 hours, the noninfective period persisted for 4-8 hours.

1 The WHO/MAL series has been chosen as a vehicle for issuing abstracts or translations in English of papers on malaria published in the Chinese medical and scientific press as most of this material is not readily available to interested readers outside China. The numbering of the abstracts in this document is consecutive to that of the abstracts given in the previous WHO/MAL/88.1047.

This paper describes some of the biological characteristics of Plasmodium falciparum from different regions of China, including their growth diversity, drug sensitivity, isoenzyme variation and indirect fluorescent reaction with monoclonal antibodies. Four cryopreserved isolates were recovered and maintained in continuous in vitro culture by the Trager & Jensen method. Two of these isolates, FCC-4/HN and FCC-5/HN from Hainan, showed rapid adaptation to cultivation and had a multiplication rate of 8-10 fold per 96 hours; the other two, FCC-102/JS from Jiangsu and FCC-101/AH from Anhui, needed an adaptation period of one month or longer before they grew stably in culture and had a multiplication rate of only 4-6 fold per 96 hours. Except for FCC-102/JS, the isolates adapted easily when the culture medium was changed from human serum to rabbit serum. Gametocytes were found in all the cultures; gametocytogenesis was stronger, however, for the parasites from Jiangsu and Anhui, one of which, FCC-102/JS, still had a high gametocyte rate of 10% after about 8 months in culture.

The 48-hour in vitro micro-test for drug sensitivity showed that the FCC-1/HN, FCC-101/AH and FCC-102/JS isolates were sensitive to chloroquine with a mean 50% effective concentration (EC50) of 46.86 ± 4.67 nmol/l, and that the FCC-4/HN and FCC-5/HN isolates and the Cambodian I strain were resistant to the drug with a mean EC50 of 246.36 ± 34.56 nmol/l.

Isoenzyme variation was detected by polyacrylamide gel slab electrophoresis. Two types (fast and slow) of 6-phosphogluconate dehydrogenase (6PGD) and of glucose-6-phosphate isomerase (GPI) were detected in the isolates from Hainan and Cambodia, but only the slow band was observed for the parasites from Jiangsu and Anhui. Lactate dehydrogenase (LDH) and glutamic acid dehydrogenase (GDH) showed a single band for all the isolates, but the mobility of GDH in FCC-101/AH was slower than that in the other isolates.


Daxin County, situated in the south-western part of Guangxi, has a population of more than 32.8 thousand. In 1957 the malaria parasite rates were as follows: 22.1% in the hilly district where Anopheles minimus was the main vector and 4.6% in the plain district where Anopheles sinensis was the main vector.

Starting in 1958, DDT indoor residual spraying and antimalarial drug administration were used as control measures. By 1979 the malaria morbidity had dropped to 0.2% and the parasite rate to 0.03%. Malaria was almost eradicated in those districts where Anopheles sinensis was the main vector, but in other districts where Anopheles minimus was the main vector many foci still existed. The antimalaria project in those districts was therefore strengthened by indoor residual spraying, case detection, presumptive treatment, radical treatment and tracing of malaria cases. After three years, all the malaria foci had been eliminated, the annual blood examination rate of the febrile patients was about 11.5%, the annual parasite incidence was down to 0.1%, quartan malaria and falciparum malaria had disappeared and tertian malaria had been decreased. In Encheng village, under malaria surveillance from 1980 to 1985, 11 256 febrile patients were examined with only two being found positive, and of 903 infants and 6806 inhabitants examined, none had the malaria parasite.

The study of some biological and clinical aspects of *Plasmodium vivax* in Yunnan and Hunan Provinces showed that:

1. The developmental period of *P. vivax* in mosquitoes was 9 days at a temperature of 26 ± 1°C. Parasitaemia occurred from 8 to 13 (9.9 ± 1.3) days after exposure in Yunnan, 11 to 16 (14.3 ± 1.6) days after exposure in Hunan. No pronounced correlation between the length of the prepatent period and the number of sporozoites inoculated was found.

2. In most of the cases artificially infected with *P. vivax* the clinical attack occurred in the afternoon and the typical tertian fever was seen only in 2 of 14 patients. The natural course of infection was as short as 9 to 14 days in nonimmune cases.

3. Mosquitoes fed at the time when the parasite was first detected following sporozoite inoculation did not become infected; but the infection rate in mosquitoes fed on patients at the first onset was 7.9% in Yunnan and 95.0% in Hunan. Such a great difference in infectivity was briefly discussed in terms of parasite biology.


As artemether and artesunate are both soluble in water, they do not present the difficulties in preparation caused by the insolvibility of artemisinin. The treatment of 772 patients with various forms of malaria at a total individual dose of 160-800 mg yielded a 100% recovery rate and the parasite clearance time was shorter than that of artemisinin and six other antimalarials. In particular, the treatment of 25 severe cases of malaria, with either intramuscular or intravenous injection of sodium artesunate led to satisfactory results. It is the most effective new antimalarial drug. There were no side-effects, and no significant influence on the function of heart, liver, kidney and other important organs. However, the recrudescence rate of 46.6% (304/652) was relatively high.


Ten cases of symptomatic chloroquine-resistant *falciparum* malaria in Hainan Island were tested with the WHO 28-day in vivo observation method. These cases included 9 nonimmune immigrants and one 12-year old local child. Three cases (2 RII and 1 RIII) were treated with a single intramuscular dose of 160 mg pyronaridine phosphate. Seven cases (2 RI, 2 RII and 3 RIII) were treated with 480 mg pyronaridine phosphate in 3 days (160 mg intramuscular dose daily). The average defervescence times for the 160 mg group and the 480 mg group were 26.7 ± 16.2 h and 30.3 ± 22.6 h respectively. The mean asexual parasite clearance times for these two groups were 50.6 ± 18.9 h and 56.4 ± 20.8 h respectively. Follow-up studies revealed that all of the 3 cases in the 160 mg group recrudesced, the average time of recrudescence being 13.7 days, while the 7 cases in the 480 mg group were all cured. This study indicates that the dosage of pyronaridine phosphate to be used in the treatment of chloroquine-resistant *falciparum* malaria should not be lower than 480 mg or 9 mg/kg body weight.

The minimum effective concentration (MEC) of pyronaridine against *Plasmodium falciparum* FCC1 was determined by an *in vitro* 48-h assay using serum from rabbits previously dosed with pyronaridine. It was found that the MEC in assays with dosed rabbit serum was 4.7 ± 1.2 ng/ml and in those with control rabbit serum, to which pyronaridine was added, was 5.2 ± 2.0 ng/ml. There was no significant difference between the two values (p>0.05). On the basis of these data together with pharmacokinetic data from malaria patients studied previously, it is proposed that the following dosage regimens be applied for the use of pyronaridine in the treatment of falciparum malaria: the intramuscular administration of 4 mg/kg body weight on day 1, followed by 2 mg/kg on day 2; or the oral administration in capsule (or enteric coated tablet) form of 10 mg/kg on day 1, followed by 5 mg/kg on day 2. It is suggested that these new regimens would maintain the blood concentration of pyronaridine at a level of about two times the MEC for a period of up to 72 h after the first intramuscular injection or after the first oral dosing. However, further clinical studies should be conducted to evaluate the efficacy and tolerance of these new regimens.


Primaquine either alone or in combination with pyronaridine was administered intragastrically to mice. The number of mice that died in the treatment groups given pyronaridine at a dosage of 293-507 mg/kg body weight combined with primaquine at a dosage of 50 mg/kg was not higher than that in the group given primaquine alone and was significantly lower than that in the groups given a combination of chloroquine at a dosage of 102-253 mg/kg and primaquine. Primary tissue schizontocidal activity was evaluated in rhesus monkeys inoculated with *Plasmodium cynomolgi* sporozoites. Results showed that all monkeys were cured by treatment with either primaquine alone administered intragastrically at a dosage of 3 mg/kg/day x 3, or a single intramuscular dose of 10 mg/kg of pyronaridine combined with primaquine starting on the day of infection. Finally, no influence of pyronaridine on primaquine was observed in *P. yoelii* sporozoite-infected mice.


It has been reported that ketotifen, cyproheptadine and pizotifenum can cure mice infected with *Plasmodium yoelii* and that ketotifen and cyproheptadine can cure monkeys infected with *P. cynomolgi*. In the present study observations were made on the inhibitory effect of the above-mentioned three drugs *in vitro* on the development of *P. falciparum*. Concentrations of 5 x 10^{-5} M of ketotifen and 1 x 10^{-4} M of cyproheptadine and pizotifenum inhibited the trophozoites from developing into schizonts; the trophozoites became degenerated 30 hours after drug exposure.


The *Plasmodium yoelii yoelii-Anopheles stephensi* system was chosen as experimental model for studying the effects of pyrimethamine on oocyst formation of *Plasmodium* species. The drug was given by allowing mosquitoes to feed on infected and pyrimethamine treated mice or by feeding them directly with a pyrimethamine-sugar water solution. The infective rate and the number of oocysts formed after drug administration were both
reduced. Moreover, the oocysts formed were smaller and their daily growth rate slower than that of the controls. Electron microscopic studies using the Feulgen staining method showed that the cytoplasm of the affected oocysts contained many vacuoles, pigment aggregations and black aggregates. No nucleus was apparent in the affected oocyst, which had presumably deteriorated to become a "black spore". The amount of DNA in drug-affected oocysts was scanty. No sporozoites were found in the salivary glands of these mosquitoes. It was suggested that pyrimethamine interfered with DNA synthesis of oocysts.


A study was made of the effect of nitroquine acetate, together with pyrimethamine, on the incorporation of \(^{3}H\)hypoxanthine into DNA and RNA of Plasmodium yoelii during incubation in Men Eagle medium containing neither para-aminobenzoic acid (PABA) nor folic acid. Incorporation into RNA was only slightly affected by nitroquine at a concentration of 10 \(\mu\)mol/l, whereas incorporation into DNA was markedly inhibited by nitroquine at 0.1 \(\mu\)mol/l and by pyrimethamine at 1 \(\mu\)mol/l. When both drugs were at 10 \(\mu\)mol/l, inhibition of DNA synthesis was noted after 15 minutes of drug action. The inhibition was antagonized by PABA, folic acid and folinic acid when both antimalarials were at 10 \(\mu\)mol/l. In the absence of antimalarials, incorporation into DNA was stimulated by the three antagonists. The study results indicate that nitroquine, like pyrimethamine, inhibits DNA synthesis of malaria parasites by interfering with their folic acid metabolism, which is one of the main antimalarial mechanisms of nitroquine.


A technique for assessing the in vitro growth of Plasmodium falciparum by fluorometry was used to evaluate the antimalarial efficacy of artemisinin, arteether and chloroquine. After cultivation in various concentrations of the above drugs for 40 hours, parasitized red blood cells (RBC) were harvested, lysed, stained with ethidium bromide, solubilized with sodium dodecyl sulfate (SDS), and measured by a fluorescence spectrophotometer. The 50% inhibition concentrations (IC\(_{50}\)) of the three drugs determined by fluorospectrophotometry were closely related to those obtained by visual counting of schizont-infected RBC in thin blood films under the microscope. Currently used methods can be supplemented by this new technique which offers the following advantages: (1) a more objective reading, and (2) a simpler and safer procedure than the radiometric assay.


A study was made of the electrophoretic behaviour of spleen lymphocytes and lymph-node lymphocytes from normal mice, mice infected with Plasmodium yoelii, and mice that were twice infected with P. yoelii at a 5-day interval and that recovered spontaneously 21 days after the infection.

Normal mouse spleen lymphocytes displayed two electrophoretic peaks: one moved at a mobility of 1.3 u/S/V/cm and the other at a mobility of 0.5-0.6 u/S/V/cm. Lymphocytes from the spleens of infected mice appeared to move towards the fast peak which contained mainly T cells while those from the spleens of spontaneously recovered mice moved towards the slow peak which consisted chiefly of B cells. Lymphocytes from the lymph nodes of mice belonging to the above-mentioned groups showed mobility variation similar to that of the spleen lymphocytes. The mechanism underlying the phenomenon was discussed briefly.

A study on the behaviour of erythrocytes during invasion by rodent malaria merozoites was carried out in vivo using a transmission electron microscope (TEM). It was observed that the shape of the erythrocytes underwent various changes even before their contact with the parasite. Basically, there were two types of deformation, i.e. cup-like and pseudopod-like. In the first type, the erythrocyte formed a concavity on the part adjacent to the merozoite so as to embrace the parasite. In the second type, the erythrocyte produced protrusions or pseudopods so as to capture and surround the merozoite. During the middle and late stages of the invasion, the red cells exhibited an obvious forward-advancing posture toward the merozoite by either raising the concavity or tapering the invagination wall as wavy projections. These morphological phenomena suggested that the red cells were so strongly stimulated in their endocytic activity by the merozoites that they were able to conduct amoeboid movement to enclose the merozoites. In this way, a parasitophorous vacuole might be formed and incorporation of the parasite realized.


The effects of glycophorin A (GPA), anti-GPA IgG, a1-acid glycoprotein (a1-AGP), ovomucoid (OM) and wheat germ agglutinin (WGA) on the invasion of erythrocytes by Plasmodium falciparum were observed in vitro. GPA, anti-GPA IgG and WGA at low concentrations had an obvious inhibitory effect on the invasion. There was an hyperbolic relationship between the concentrations of these inhibitors and their inhibition rates. OM, a1-AGP and non-specific anti-serum had no obvious effect on the invasion. It is confirmed for the first time on the basis of the biological characteristics of receptor-ligand interaction that the combination of GPA with P. falciparum merozoites has high specificity, high affinity with a tendency to saturation and can produce specific biological effects.


Glycophorin A and glycophorin B of the human erythrocyte membrane were purified by chloroform-methanol extraction followed by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). These molecules were then tested in an in vitro inhibition assay for their ability to inhibit invasion of human erythrocytes by Plasmodium falciparum merozoites. Glycophorin B showed 34% and 50% inhibition at 150 µg/ml and 200 µg/ml, respectively. Glycophorin A showed 91% inhibition at 150 µg/ml. These results suggested that both glycophorin A and B were involved in the recognition and invasion of human erythrocytes by P. falciparum merozoites.


Serum samples were collected from mice immunized separately with schizonts, merozoites or different parasite stages and from mice which had recovered from Plasmodium yoelii yoelii infection. The effect of protection of each serum against P. yoelii infection was tested by the passive transfer technique. Only sera collected from mice which either had been treated with chloroquine or had spontaneously recovered from P. yoelii infection were able to transfer a certain degree of resistance to normal mice. These "protective" sera delayed the appearance and the peak of parasitaemia and prolonged the survival time of challenged mice. These sera were fractionated by
50% \((\text{NH}_4)_2\text{SO}_4\) precipitation, and the precipitated fraction showed a similar degree of protection as the non-fractionated serum. It was shown that chloroquine-treated serum was able to inhibit the invasion of new erythrocytes by merozoites in vitro.

\(^{35}\text{S}\)-methionine-labelled antigens were precipitated by either "protective" or "non-protective" sera and the bands were compared on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiogram. Since antigens with relative molecular weights of 245 000, 210 000, 190 000, 156 000 and 130 000 can only be precipitated by "protective" sera and not by "non-protective" sera, it is possible that the protection conferred by serum from chloroquine-treated mice is induced by the specific antibodies against the above antigens.


Antigens extracted from the blood stage of \textit{Plasmodium cynomolgi} which was derived from either \textit{in vitro} culture or infected monkey were compared in experiments using the enzyme-linked immunosorbent assay (ELISA) to detect antibody to vivax malaria. Among 64 cases with vivax malaria a positive rate of 96.9% was obtained 19-90 days after the onset of a malaria attack with both antigens and the titres for the two antigens were quite similar. However, mature parasites can be more readily collected from \textit{in vitro} cultures and the antigen extracted from them can be freshly prepared thereby overcoming the problem of loss of reactivity upon storage. The usefulness of polyvinyl chloride (PCV) films to replace traditional plates and of the innocuous tetramethylbenzidine salt (TMBS) as a substrate for horseradish peroxidase (HRP) was again confirmed by these ELISA studies for the detection of antibodies to vivax malaria.


The correlation between the immunofluorescent properties of the monoclonal antibodies against \textit{Plasmodium falciparum} and their protective activities was investigated. Monoclonal antibodies which reacted with surface antigens of free merozoites and segmenters to produce spot-like or pin-like fluorescence as well as honeycomb-like bright fluorescence caused inhibition of growth, opsonization and cytotoxicity in \textit{Plasmodium falciparum}. This effect was not related to antibody levels as measured by the indirect fluorescent antibody test (p>0.05).


1299 patients with vivax malaria were followed up by the indirect fluorescent antibody (IFA) test and by blood examination in a malarious area where antimalaria measures had been applied from June 1980 to March 1981. The patients were divided into two groups: group 1 consisted of 1110 patients treated with two courses of chloroquine 3 to 4 days after the onset of malaria; group 2 consisted of 189 patients treated irregularly in terms of time and/or dose regimen. Both groups had similar antibody levels within the first four weeks after falling ill. One month later, the antibody positive rate and the geometric mean reciprocal titre (GMRT) in group 1 declined rapidly as opposed to group 2 (p<0.05). Resurgence of parasitaemia was seen more frequently in group 2. The six-month cumulative parasite positive rate was 2% for group 1 and 33.9% for group 2. At 3 and 6-9 months after illness, the IFA positive rate dropped to 34.6% and 11% respectively in group 1 and to 66.7% and 24.2% respectively in group 2.

The 879 patients who became free of malaria within a year were subdivided into three age groups: 15 years or under, 16-30 years and 31 years or over. The differences in GMRT among the three age groups were very small (p>0.05). One month after illness, the
antibody negative-conversion speed slackened in the older age groups. The antibody positive rates for the three age groups at the 4-6 month follow-up were 15.3%, 15.5% and 34.8% respectively and at the 6-9 month follow-up they were 9.1%, 6.1% and 14.4% respectively.

Blood film examination revealed that 67% (56/84) of the parasite carriers were antibody positive and that the antibody positive rate was related to the intensity of parasitaemia while the antibody titre was not.