

HUMAN MALARIA IN NON-HUMAN PRIMATES: EXPERIMENTAL MOSQUITO TRANSMISSION AND INFECTION*

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The susceptibility of splenectomized white-handed gibbons (*Hylobates lar lar*) and macaque monkeys (*Macaca irus*, *M. mulatta* and *M. nemestrina*), to blood-induced infections of *Plasmodium falciparum* was reported previously by Ward *et al.* (1965), Ward and Cadigan (1966), Cadigan *et al.* (1966). Susceptibility of the splenectomized gibbon to sporozoite-induced falciparum infections was reported by Gould *et al.* (1966). The present report describes further experiments in mosquito transmission and mosquito infection with falciparum and vivax malaria in the above hosts and in the tree shrew (*Tupaia glis*).

MATERIALS AND METHODS. These investigations were conducted in Phrabuddhabat, Saraburi Province, Thailand at the U. S. Army Medical Component, SEATO facility on the grounds of Headquarters Region I, Thai National Malaria Eradication Project, from August 1965 to June 1967. The animals used were splenectomized and given a course of radical treatment with chloroquine and primaquine by the Department of Veterinary Medicine as described by Ward *et al.* (1965) prior to inoculation with the sporozoites or blood forms of *P. falciparum* or *P. vivax*. They were housed in a screened building at all times. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

The mosquitoes used were colonized strains of *Anopheles balabacensis* Baisas and *A. stephensi* Liston, originating in Thailand and India, respectively. Sporozoites of *P. falciparum* and *P. vivax* were produced by feeding these mosquitoes on

gametocyte carriers seeking treatment at the passive detection center, Headquarters Region I, Thai National Malaria Eradication Project. Sporozoite inoculations were accomplished by direct feeding, or, in order to increase the dosage, by inoculating the ground mosquitoes intramuscularly and intra-peritoneally. In the latter case, the mosquitoes were ground with a Tenbroeck grinder in normal saline solution, in 50 percent human serum in normal saline solution, or in Ringer's solution. Presence of sporozoites in salivary glands was determined by dissection of an aliquot of mosquitoes prior to inoculation, or by microscopic examination of a sample of the inoculum.

Mosquito feedings on the animals were accomplished by restraining the animal on a holding board and allowing the mosquitoes to feed through the screened end of a cylindrical plastic feeding cage for 1 or 2 hours. As a rule, dissections were made for the oocyst stage 4 to 8 days following the experimental feeding; however, due to the press of circumstances, dissections were frequently postponed, and it was then necessary to dissect for the sporozoite stage or for both stages. Pending dissection mosquitoes were held in an insectary at approximately 27° C. and 80 percent relative humidity.

Blood examinations were made on Giemsa-stained thin films and leucocyte counts were made by standard procedures with the Spencer "Bright-Line" hemacytometer. Gametocyte counts per cubic millimeter of blood were computed on the basis of the leucocyte counts and rounded off to two significant figures.

RESULTS. Sporozoite Inoculations. Table 1 summarizes the results of attempts to infect animals by sporozoite inoculation made at the Phrabuddhabat laboratory.

* This paper is contribution number 250 from the Army Research Program on Malaria.

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TABLE 1.—Summary of results with sporozoite-inoculated animals.

Animal	Malarial Species	No. of Trials	No. of Animals	No. of Successes	Prepatent Periods (Days)
Gibbon	<i>P. falciparum</i>	10 ¹	7	3	44, 46, 70
Gibbon	<i>P. vivax</i>	2	1	0
Macaque	<i>P. falciparum</i>	4	2	0
Macaque	<i>P. vivax</i>	1	1	0
Tree Shrew	<i>P. falciparum</i>	3	2	0

¹ Includes three inoculations of frozen sporozoites furnished by Dr. R. D. Powell, University of Chicago, Chicago, Ill.

Success has been achieved only in the inoculation of gibbons with *falciparum* sporozoites. Two previous sporozoite-induced infections were reported by Gould *et al.* (1966). In a third successful attempt, the gibbon became patent after 70 days, and remained intermittently patent until day 246 following inoculation. In this animal peak parasitemia (1500 parasites per 500 leucocytes) and gametocytemia (55 gametocytes per 500 leucocytes) occurred on day 126.

Mosquito Feedings on Infected Animals. No gametocytes were seen in blood-induced infections of macaques and tree shrews, and therefore no mosquitoes were fed on these animals. Sporozoite-induced and blood-induced *falciparum* infections in the gibbon are essentially similar except that in the latter (a) the exoerythrocytic stages are presumably absent; (b) the prepatent period is shorter, commonly being 2 to 20 days; (c) parasitemias are much higher in the first passage, commonly exceeding 10⁵ parasites per cubic millimeter; and (d) it is possible to further increase parasitemia levels of *falciparum* strains in the gibbon by serial blood inoculations. All mosquito feeding trials were done on blood-induced *falciparum* infections.

The highest gametocytemia observed during the feeding trials was estimated at 200,000 gametocytes per cubic millimeter of blood. *Falciparum* gametocytes in the gibbon are characteristically quite small, ranging from 3 to 9 microns in length as compared with 7 to 15 microns in human cases. The smaller ones are elliptic or elliptic-acuminate in shape, and the larger

ones are usually falcate. The typical sausage-shape with rounded ends is extremely rare; only three such gametocytes have been seen in detailed examinations of more than forty blood films from gametocytemic gibbons. The pigment in gametocytes from the gibbon is coarser and more rod-like than the small granules seen in human cases. The small gametocytes seen in the gibbon resemble, in size and shape, the immature gametocytes described from human cases, which are rarely found in the peripheral circulation. Likewise, it is not unusual to see advanced or late stage trophozoites and schizonts in blood films of *falciparum*-infected gibbons.

The life span of *falciparum* gametocytes in human cases reportedly extends to as long as 20 days (Thomson, 1911), and it is not unusual to find *falciparum* gametocytemia without an accompanying asexual parasitemia in human cases. In the gibbon, on the other hand, continuous gametocytemia exceeding 4 days' duration was unusual and in only one case were gametocytes seen without accompanying asexual forms.

All attempts to infect mosquitoes by feeding on gametocytemic gibbons gave negative results. Briefly, there were 44 feedings on 24 individual gibbons carrying six isolates of *falciparum* malaria in the first to twentieth gibbon passage. Gametocytemias up to 200,000 per cubic millimeter were observed. Six gibbons were used on two or more days, at different stages of their infections. There were consecutive daily feedings of 2 days on each of two gibbons and of 3 and 5 days on a

third animal. Seven feedings covering a 24-hour period made at 4-hour intervals were also done on the latter animal. In all, 988 mosquitoes of the two species were dissected. Both mosquito strains were demonstrated to be susceptible to *falciparum* malaria by concurrent feedings made on human cases.

Included in the above trials were several special experiments intended to enhance our chances of achieving mosquito infection (Table 2). Each experiment included

connection with three different feeding trials. As a check on the method, the procedure was repeated a fourth time with mosquitoes fed upon a human case of *falciparum* malaria. Each of the stained films from mosquitoes fed on infected gibbons was searched for ookinetes under the oil-immersion lens for 30 minutes. No forms resembling the ookinete were found. A great many of the gametocytes seen were deformed and appeared to be degenerate. Gametocytes became progressively fewer

TABLE 2.—Summary of special experiments in feeding anophelines on *falciparum*-infected gibbons.

Procedure	No. of Trials	No. of Mosquitoes Dissected
Post-feed incubation of mosquitoes at 22° C	3	23
Post-feed incubation of mosquitoes at 32° C	2	8
Pre-feeding mosquitoes at one week on normal gibbon	2	7
Pre-feeding mosquitoes at one week on normal human	2	10
<i>In vitro</i> feeding of infected blood diluted to ½ with normal gibbon blood	2	13
<i>In vitro</i> feeding of infected blood diluted to ½ with normal human blood	3	18
<i>In vitro</i> feeding of infected blood, plasma replaced with normal human plasma	2	61
<i>In vitro</i> feeding of infected blood, 5 percent (w/v) glucose added	4	25

appropriate controls, but these are omitted from the table.

Experiments were also undertaken to determine at what stage *falciparum* gametocytes from gibbons fail to complete their development following ingestion by the mosquito. Exflagellation was observed in four of six feeding trials in which it was sought in slow-drying thin blood films and fresh cover-slip preparations of finger blood from gibbons and blood expressed from the stomachs of mosquitoes freshly fed on gametocytemic gibbons. Subsequently, exflagellation was observed again, quite incidentally, in an ordinary thin blood film prepared during another feeding trial.

The ookinete stage was sought by the examination of thin blood films prepared from the stomach clots of mosquitoes 10 hours after the feeding trial, and at 2-hour intervals thereafter up to the 24th hour. This procedure was done three times in

in number in successive blood films and at 24 hours after mosquito feeding it was very difficult to find any. In smears from mosquitoes fed upon the human case, unmistakable ookinetes were observed on the blood film made 16 hours after mosquito feeding and in those prepared thereafter; deformed gametocytes were relatively uncommon in these smears and there was no noticeable reduction in gametocyte numbers in the successive films.

DISCUSSION AND SUMMARY. There have been few successful infections of non-human primates with human malaria by sporozoite inoculation. In the experiments described here, attempts were made to infect white-handed gibbons (*Hylobates lar lar*), macaques (*Macaca irus*, *M. mulatta* and *M. nemestrina*) and tree shrews (*Tupaia glis*) with sporozoites of both *P. falciparum* and *P. vivax*. Infections were established only in three gibbons inoculated with *P. falciparum* sporozoites. While

failures in the case of the other primates tested may have been a function of the sporozoite dosage employed, it was not possible in these studies to obtain the necessary numbers of infected mosquitoes or experimental animals to test this hypothesis.

The prepatent periods in the three gibbons infected with *P. falciparum* by sporozoite inoculation were 44, 46 and 70 days, respectively. In studies of avian malaria, the pre-patent period has been widely used as an index of the host-resistance (Huff, 1963). By this criterion the prepatent periods observed in the gibbons would suggest that this host is significantly more resistant to infection with *P. falciparum* than are human beings.

Garnham (1966) observed that where malarial parasites have been established in unnatural hosts the gametocyte stages have either been non-infective for mosquitoes or absent. Our experience with *falciparum* gametocytes in splenectomized gibbons has borne this out, for in 44 attempts to infect anophelines by feeding on gametocytemic gibbons no infections were established in mosquitoes. The microgametocytes in gibbons infected with *P. falciparum* were found to be capable of exflagellation, but formation of the zygote and development to the ookinete stage were not achieved.

In view of the foregoing discussion, the potentiality of the gibbon as a natural reservoir of *falciparum* malaria seems to be nil.

ACKNOWLEDGMENTS. The authors wish to thank Dr. Bhavongvit Tantichareon without whose cooperation and assistance this study would not have been possible.

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