

STUDIES WITH INDUCED MALARIAS IN *AOTUS* MONKEYS^{1,2,3}

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SUMMARY • *Aotus* monkeys have been shown to be susceptible to the three most important species of human malaria as well as to simian, ape, and rodent malaria. The parasites natural to man were maintained by serial passages using trophozoite infected blood. It was then possible to transfer the infections to, and among, other New World monkeys which previously were refractory. The parasites retained their viability in the monkey, were infective to mosquitos, and were transmitted between monkeys and back to man. Exoerythrocytic stages, similar to those demonstrated in man, occurred in the *Aotus* liver. Drugs exerted similar action against the parasites in the monkey as against parasites in man. The human malaria-*Aotus* model has made possible other extensive studies in various disciplines important to the understanding of the parasites in man. These included parasite biology, host-parasite-vector relationships, immunology, pathology, and physiology.

KEY WORDS • Malaria—Animal model—Owl monkey

During the middle 1960's it became apparent that in widespread areas of the world, strains of *Plasmodium falciparum*, a highly pathogenic parasite, were resistant to the best clinical drugs represented by the 4-aminoquinoline group of compounds (1). An intensive search for new drugs was begun. One of the needs of such a program was a convenient inexpensive laboratory animal in which human malaria could be grown. None was available. The only animal hosts were the large apes. For a number of reasons, they were not practical for wide scale use. As a consequence, a project was initiated at the Gorgas Memorial Laboratory, with US Army Research and Development support, to determine if Panamanian monkeys would serve as models.

Early in the program, it was shown for the first time that *P vivax* could develop and be maintained in New World monkeys. The initial successful host system was *Aotus trivirgatus* (2). Subsequently, this was confirmed by several workers (3-5).

It has been found that two other species of human plasmodia, namely *P falciparum* (3) and *P malariae* (6), as well as other primate malarias, are infective for these monkeys.

In the present report, some of the results of the various investigations, especially human malaria in *Aotus*, will be considered.

PARASITE ADAPTATION

Most of the attempts to infect *Aotus* with *P vivax* have been successful. At first we used immunosuppressant drugs, with and without splenectomy. These alterations appeared to help, but later it became unnecessary to modify the monkeys. It appeared that serial passage increased the infectivity of the malarias. Also, fewer parasites were required in the inoculum.

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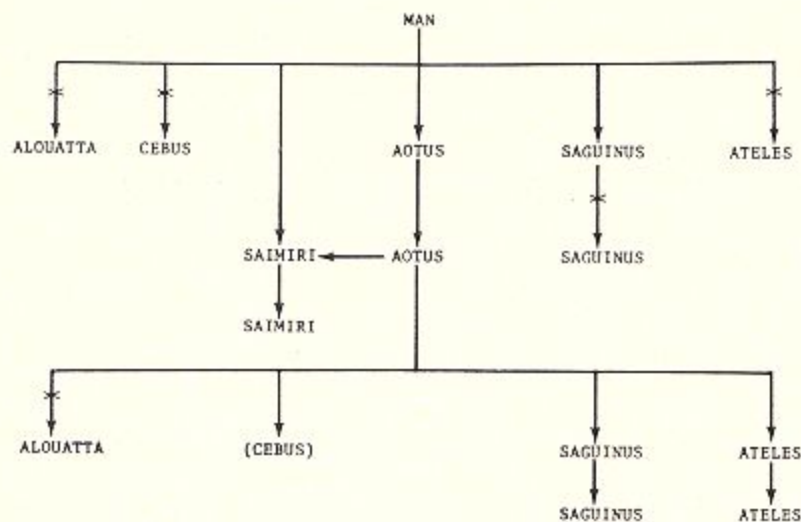


Fig 1. Adaptation and passage of *Plasmodium vivax* in New World monkeys. —

There was other evidence of a change in the parasites. In the beginning, we were not able to establish passage in *Ateles* and *Saguinus* when the parasites came directly from man (Figure 1). However, after becoming adapted in *Aotus*, passage lines could be initiated in these two genera, with transfer among them and *Aotus*. As shown, *Saimiri* also was highly susceptible, whereas others, *Alouatta* and *Cebus*, remained refractory.

Strains of *P. vivax* (originating from at least eight countries on four continents and from widely separated areas) have been readily maintained by passage of infected blood. Occasionally, sporozoite transmission has been interspersed where possible. The Achiote strain of Panamanian origin has been serially passed 136 times over a 9-year period.

Plasmodium falciparum shows a more precise host specificity (Figure 2). Many trials were made in Panama to infect *Aotus* monkeys, and indeed other species of monkeys, with malarious blood direct from man (7). We were able only to produce a few transient parasitemias, without sustaining a passage line, using indigenous monkeys. In contrast, others using a Panamanian strain of *P. falciparum* were able to obtain passage

lines in *Aotus* monkeys from Colombia, a neighboring country (8). Once adapted to Colombian *Aotus*, the parasites were then infective for Panamanian *Aotus*. Undoubtedly, the parasite had been modified.

Also, the *Aotus* monkey (from Colombia) has been infected with *P. falciparum* from diverse areas of the Old World (for example, Africa and Southeast Asia). Whether these strains can be induced in the Panamanian *Aotus* monkey directly from man is not known. The local *Aotus* can be readily infected with several of these isolates as indicated in Figure 2, by using infected blood from the Colombian *Aotus*. Other species of New World monkeys then show marginal to good susceptibility.

Of considerable importance is that *P. falciparum* strains, either sensitive or resistant to chloroquine and pyrimethamine, can reproduce in these systems (9). This has aided in evaluating the potential of new compounds and in comparing the response to those in man.

After a number of passages of the human malarial the parasitemias increase in density. They often will exceed by several times the densities of the same species occurring in man. *P. vivax* infections can kill these mon-

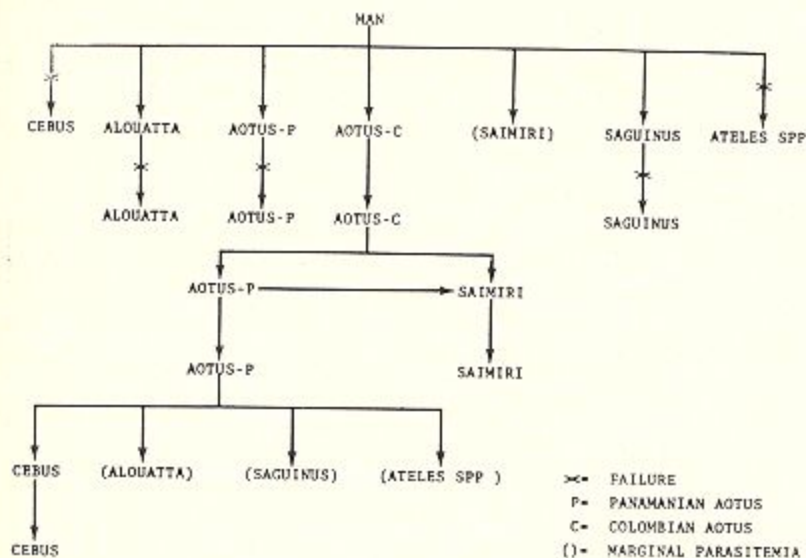


Fig 2. Adaptation and passage of *Plasmodium falciparum* in New World monkeys.

keys, a result seldom seen in man. The parasitemias of *P. falciparum* reach much higher levels than in man and they are also fatal to the monkey.

Few attempts have been made to infect *Aotus* monkeys with *P. malariae* and *P. ovale*, and thus far parasitemias (which also reach lethal densities) have been produced only with the former species (10).

VECTOR INFECTIONS

Many natural mosquito vectors have been tried with these hosts, and great differences in rates of infection have been observed (11-13). *Anopheles albimanus* is the best of the Panamanian mosquitoes for *P. vivax* malaria in *Aotus*, but comparatively it was not consistent and the infections were often of a low grade. Among other mosquitoes tested, *A. freeborni* from the USA was the most efficient. Additional species that have shown good utility are *A. balabacensis* from Southeast Asia (5) and *A. stephensi* from India (14).

The period of infectivity of the gametocytes may be erratic. Often, they will infect mosquitoes for only 1 day, or on a few wide-

ly separated days during the patient period. *Plasmodium vivax* infections in man are more predictable. Usually a human patient could infect mosquitoes for a number of consecutive days.

It is more difficult to infect mosquitoes from the monkey than from man with the same strain. However, it appears that the vector infectivity of *P. vivax* lines may be improving in our laboratory. Recently, some excellent infections have been obtained. Infectivity of the gametocytes to mosquitoes has remained after many blood induced passages in monkeys. At the 105th monkey passage, mosquitoes that were infected could transmit the infection to man. This was in contrast to the failure of the first attempt to transmit a *P. vivax* infection from monkey to monkey, although transmission to man was accomplished (2). Perhaps this indicates a better adjustment of the *P. vivax* strain to the monkey and insect hosts.

There are striking variations in the infectivity of *P. falciparum* malaria in *Aotus* monkeys to both exotic and indigenous strains of mosquitoes. *Plasmodium falciparum* in Panamanian monkeys has been poorly infective for Panamanian mosquitoes (7). A Cambodian strain rarely infected the

co-indigenous *A balabacensis* and the California *A freeborni* (15), while the Malayan IV strain of *P falciparum* infected the co-indigenous *A balabacensis* and, more heavily, *A freeborni* (16). This is an interesting example of malaria from the opposite side of the world (Malaysia) growing in the *Aotus* monkey from South America and infecting healthy mosquitoes (*A freeborni*) from California. It seems unlikely that this combination has ever been associated in nature, but when all were put together, an effective combination resulted.

This is in contrast to some of the earlier findings on the infectivity of *P falciparum* in man to vectors. Working with *P falciparum* from Panama and the southeastern United States, it was shown that co-indigenous *A albimanus* mosquitoes became better infected with a co-indigenous strain of malaria than with strains originating at a distance (17). These differences in host-parasite-vector relationships for *P falciparum* offer interesting possibilities for research.

Plasmodium malariae in *Aotus* was infective to five species of mosquitoes (10). *Anopheles freeborni* was very susceptible, becoming infected from early parasite patency to the 108th day of patency. Transmission to four men occurred.

NONHUMAN MALARIA

Although the *Aotus* monkey has never been found with a natural infection of simian malaria, it is susceptible to the two monkey malaras from South America, that is, *P brasilianum* (10, 18) and *P simium* (19), as presented in Table 1. Serial passages have been demonstrated with both species, yielding especially high parasitemias for *P simium*. Transmission of both parasites was accomplished, but the tissue stages in *Aotus* have been reported only for *P simium* (20). Whereas *A freeborni* were infected with *P simium* in *Aotus*, it appeared that this host represented a "dead end" for *P brasilianum*

TABLE 1

Experimental plasmodial infections in the owl monkey, *Aotus trivirgatus*

<i>Plasmodium</i> species	Patent infection	Serial passage	Infective for mosquito	Exoerythrocytic stages
<i>vivax</i>	+	+	+	+
<i>falciparum</i>	+	+	+	+
<i>malariae</i>	+	+	+	
<i>ovale</i>	-			
<i>simium</i>	+	+	+	+
<i>brasilianum</i>	+	+	-	
<i>knowlesi</i>	+	+		
<i>fragile</i>	+			
<i>hylobati</i>	-			+
<i>pitheci</i>	-			
<i>berghei</i>	+			

because of absence of vector infectivity.

Aotus was shown to be susceptible to Old World macaque forms, the blood phase of *P knowlesi* (21), and more recently *P fragile* (22), by transmission. Attempts to produce patent infections using other primate malaras, *P pitheci* (10) of the orangutan and *P hylobati* (23) of the gibbon, have failed. In the latter instance, apparently normal exoerythrocytic stages were found. An interesting observation was the development of a rodent malaria, *P berghei* (24), in *Aotus*, which further attested to the low immunologic response of this host.

NEW AREAS OF INVESTIGATION

It has now been verified that a similar exoerythrocytic cycle to that in man occurs in the liver of *Aotus* when inoculated with sporozoites of the human malaras, *P vivax* (25) and *P falciparum* (26). In some cases, exoerythrocytic bodies were shown in the liver without ensuing parasitemia.

The exoerythrocytic bodies were less numerous in *Aotus* than in the natural host. Also, the prepatent periods were more irregular and often much longer than in man. It would be more helpful when studying the effects of suppressive and curative drugs if these developmental periods could be made more

uniform and similar to those in man. Perhaps with additional adaptation of these parasites to the monkey, a more predictable result can be obtained.

Malaria infections in the *Aotus* monkeys tended to react to certain standard drugs in a manner similar to the response in man. Using trophozoite induced infections, it has been shown that the response of strains of *P vivax* are similar to the response in man to the same drugs (9). We have confirmed findings that chloroquine cures *P vivax* trophozoite induced infections in *Aotus* (27). Furthermore, we have shown that 25 mg/kg of chloroquine base will not consistently cure sporozoite induced infections. Adding primaquine, 1 mg/kg of base daily for 14 days, cured the only case tried. Since the trophozoite stages are eliminated by chloroquine at the dosage used, our findings were the first chemotherapeutic evidence for the persistence of exoerythrocytic stages of *P vivax* in New World monkeys.

Plasmodium falciparum in *Aotus* monkeys is now being used as a pilot model for the identification of a number of new compounds with activity against this malaria (9, 28). The availability of human malaria parasites in this system makes possible in-depth studies that were previously hindered by various limitations. More precise work is now in progress on the mode of action of drugs and the evolution of drug resistance. Using *Aotus*, the hypothesis of chloroquine binding to high affinity drug receptor sites has been further explored (29).

A comparison of antigens of *P falciparum* in *Aotus* monkeys showed them to be the same as those in Gambian children (30). Furthermore, it was suggested that *P falciparum* strains may have some S-antigens that are strain specific and thus can serve as markers (31). Strains of *P falciparum* from different parts of Africa, as well as Malaysia, provided varying degrees of homologous and heterologous immunity (32, 33). Human immunoglobulin G from Africa gave incom-

plete protection against an Asian *P falciparum* in *Aotus* (34). Irradiated or formalized erythrocytes parasitized by *P falciparum*, when injected into *Aotus* monkeys conferred some immunity by increasing the survival rate (4, 35) or by developing more slowly upon rechallenge (36).

Pathophysiological investigations have produced very important findings related to *P falciparum* (37-40). Biochemical studies are of interest, especially the high level of glucose-6-phosphate dehydrogenase in *Aotus*, which may be significant in this monkey's susceptibility to *P falciparum* (41, 42). Other studies with this model have confirmed the need for p-aminobenzoic acid for parasite development (43). The disposition of the parasitized erythrocytes in the internal organs has been correlated to the functioning of these systems (44). Ultrastructure examination of the infected erythrocytes and host tissue, revealed abnormalities in the former that may be related to their entrapment in the capillaries (45-47).

Information has developed on the nephrotic syndrome associated with *P malariae* and *P brasilianum*, its simian counterpart (48, 49). Certain aspects of the glomerular pathology in the infected animals showed close similarities to those seen in human cases of *P malariae*.

It is anticipated that the above studies, using the *Aotus* model, will continue and that new projects will be initiated. Other New World monkeys, which are being evaluated, may also serve as companion or substitute systems. However, significant advances have been made possible only by the discovery of the utility of *Aotus* as a host for human malaria.

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