MONKEYS AS HOSTS OF HUMAN MALARIA

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by

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SUMMARY

Six species of New World monkeys were tested for their susceptibility to *Plasmodium vivax* of human origin. *P. vivax* was established and maintained by serial passage in the Panamanian night monkey (*Aotus trivirgatus*). *P. vivax* also infected the titi marmoset (*Saguinus geoffroyi*) but the parasites grew less well and were not maintained as easily in serial passages.

Lower grade infections were established in *Ateles fusciceps*, *A. geoffroyi*, and perhaps in *Cebus capucinus*.

P. vivax in *Aotus trivirgatus* was infective to, completed the cycle in, and was transmitted by *Anopheles albimanus* mosquitoes.

Plasmodium falciparum was established in the titi marmoset (*Saguinus geoffroyi*) for periods up to 15 days and in the night monkey (*Aotus trivirgatus*) for periods up to 8 days. Attempts failed in *Ateles fusciceps*, *Alouatta villosa*, and *Cebus capucinus* monkeys.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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The object of this work is to determine if human malaria can be grown in Panamanian sub-human primates and, if so, to determine biological information about the infection in these animals. Ultimately, it will be determined if such parasite-host models can be used for screening and testing new candidate drugs for anti-malarial activity.

Although the support by the grant started May 1, 1965, some pertinent preliminary experimental work was begun in January, 1965 and is included for completeness.

The presence of natural infections in the host monkeys is an important corollary to this work. Malaria in Panamanian primates has been of interest to workers at Gorgas Memorial Laboratory since 1930. Many monkeys coming into the Laboratory have been examined during this period. A revival of interest in primate malaria began in 1965. The data for the period of 1965-1966 was combined with that of the previous 35 years to discern the prevalence of natural malaria in wild-caught Panamanian monkeys. Of a total of 1994 sub-human primates, infections were found in 4 of the 7 species, as follows: Ateles fusciceps, 10.6 %; A. geoffroyi, 30.3 %; Cebus capucinus, 2.2 %, and Alouatta villosa, 4.0 %. (Porter, Johnson and De Sousa, 1966). All infections were Plasmodium brasilianum. No natural malarial infections were found in the two monkeys (Aotus trivirgatus, and Saguinus geoffroyi) which later were shown to be susceptible to the human malarias. This made the subsequent experimental work with human malaria in these monkeys less complicated.

Plasmodium vivax

Over 200 monkeys representing 6 species were tested for their susceptibility to human vivax malaria. It was shown that P. vivax will grow well in one monkey (Aotus trivirgatus) and less well in others.

Several strains of P. vivax were established in night monkeys (Aotus trivirgatus) by inoculation of malarial bloods. Two strains, the Achiote and the Santa Rosa, are being extensively studied at present. The Achiote strain has been through 23 night monkey to night monkey passages and the Santa Rosa strain has been through 16 night monkey to night monkey passages. Both splenectomized and intact

monkeys were infected but the infection died out after a few passages through intact monkeys. Efforts to establish a satisfactory laboratory infection in intact monkeys are being continued.

Table I summarizes the information on the infection attempts. In some animals the patent periods extended for 97 days, which gives adequate time to make detailed studies. The maximum parasitemia reached a higher and a later peak in night monkey to night monkey passages than in man to night monkey passages. Later night monkey to night monkey passages generally have shown higher parasitemias than earlier night monkey to night monkey passages. Efforts now are being undertaken to standardize the size of inoculum, the route of inoculation, and pretreatment, if any, for the night monkey.

Nine of 28 marmosets (Saguinus geoffroyi) were infected by inoculation of vivax bloods from man and 16 of 35 were infected by inoculation of vivax bloods from primates as shown in Table I. The infections resulting from the inoculation of primate bloods reached higher parasitemias than that from human bloods. The Emperador strain was passaged through three marmoset to marmoset passages.

Six of 12 black spider monkeys (Ateles fusciceps) and 4 of 6 red spider monkeys (A. geoffroyi) were infected with vivax bloods from primates. All infections were with the Achiote strain. The peak parasitemias were not as high in red spider monkeys as in black spider monkeys and were not as high in either as in night monkeys or marmosets infected with vivax bloods from primates. The infection has been through four spider monkey to spider monkey passages.

One of 14 whiteface monkeys (Cebus capucinus) was infected with vivax blood from a primate. The maximum parasitemia was less than 10 per cmm. and the period of patency was five days.

P. vivax was transmitted by mosquitoes from primates to two human volunteers (Table III). Anopheles albimanus mosquitoes infected from night monkeys were used in the studies. Unsuccessful attempts were made to infect four primates (one a night monkey that participated in the study with the two volunteers) by bites of infected mosquitoes

and by inoculation of sporozoites into the liver. No infections resulted nor were exo-erythrocytic bodies found in the liver on biopsy.

Plasmodium falciparum

Over 90 attempts were made to transmit P. falciparum of human origin to monkeys representing 6 species. As shown in Table II, one night monkey and six marmosets were infected with P. falciparum by blood inoculation. The maximum parasitemia in the night monkey was less than 10 per cmm. and the period of patency was eight days. The maximum parasitemia in the marmoset was 22,660 per cmm. and the maximum period of patency was 15 days. Two of the marmosets showed gametocytemias a few days subsequent to the blood inoculations. Two attempts to transfer the infection by blood to other primates failed.

Nineteen transmissions of P. falciparum to Panamanian primates by the bites of infected mosquitoes or by the inoculation of sporozoites into the liver were attempted (Table III). Infections did not occur and exo-erythrocytic bodies were not found in the liver on biopsy.

Discussion

When this work was started two years ago, the ability of monkeys, especially those of the New World, to act as hosts of human malarial parasites was only poorly known. The only success was that of Taliaferro and Taliaferro (1934), who had obtained survival of the Plasmodium falciparum parasites in the howler monkey (Alouatta villosa) for a short period of time.

We were able for the first time to establish Plasmodium vivax in several monkeys. In one (Aotus trivirgatus) this parasite grows well and is being serially transferred.

We have had partial success in maintaining P. falciparum parasites in the titi marmoset (Saguinus geoffroyi).

In most of these transmission attempts, methods to make the host more responsive to the parasites by altering the immonological response were used, such as splenectomy and immunosuppressant drugs. These appeared

to have some influence but more exact measurements of this effect are needed. Some successes in growing these malarias in unaltered hosts were obtained but the growth of the parasite appeared to be better in the altered hosts.

Anopheles albimanus, an important vector of human malaria in Panama, was found to be susceptible to the P. vivax in the monkey. The sporogonic cycle was completed in the mosquito and transmission back to the human host was successful. However, transmission from monkey to monkey has not been accomplished. This problem is receiving attention at present as the explanation of this failure might have important implications concerning the host-parasite relationships.

One of the principal objectives of the continuing work is to learn enough of the host-parasite relationships to accomplish serial mosquito transmission of these malarias in monkeys. Such is necessary to establish this parasite-host system as a model for some of the important phases of drug testing.

It is possible that another mosquito vector is better for the serial transmission of the malaria in the monkeys. Therefore, other anophelines of this area will be colonized, if possible, and their abilities as vectors tested.

Another phase of the work is the elucidation of the fixed tissue stages of the malarial cycle in the monkey. This will be an object of study when the mosquito transmission problem is solved.

The work accomplished so far indicates that additional searches should be made for other, and perhaps better, parasite-host combinations. Of particular importance will be the endeavor to establish drug resistant strains of malaria in these monkeys.

Publications

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TABLE I

TRANSFER OF *PLASMODIUM VIVAX* FROM JANUARY 1, 1965 TO APRIL 30, 1967

Species of Monkey	Man to Monkey		Inoculum 10^6	Prepatent Period	Patent Period	Parasitemia Maximum	
	Attempts	Successes*	Range	Range Days	Range Days	per cmm.	Day of Patency
Aotus trivirgatus	18	15	<1-58	1-41	5-97	80,880	15
Saguinus geoffroyi	28	9	1-142	1-46	3-37+	20,540	27
Ateles fusciceps	11	0	<1-58	0	0	0	0
Ateles geoffroyi	1	0	?	0	0	0	0
Cebus capucinus	6	0	2-64	0	0	0	0
Alouatta villosa	2	0	?-12	0	0	0	0
			MONKEY TO MONKEY				
Aotus trivirgatus	86	75	<1-134	1-24	3-93	266,710	43
Saguinus geoffroyi	35	16	<1-373	1-72	5-96	148,080	45
Ateles fusciceps	12	6	1-187	2-23	3-46	105,920	13
Ateles geoffroyi	6	4	1-52	4-12	23-56	24,350	29
Cebus capucinus	14	1	1-600	1	5	<10	3

* Demonstrable parasitemia seen for at least three successive days.
+ Continuing.

TABLE II

TRANSFER OF *PLASMODIUM FALCIPARUM* FROM JANUARY 1, 1965 TO APRIL 30, 1967

Species of Monkey	Man to Monkey		Inoculum 10^6	Prepatent Period	Patent Period	Parasitemia Maximum	
	Attempts	Successes+	Range	Range Days	Range Days	per cmm.	Day of Patency
Aotus trivirgatus	32	1	<1-156	1	8	<10	8
Saguinus geoffroyi	14	6	<1-671	1-3	4-15	22,660	2
Ateles fusciceps	7	0	<1-301	0	0	0	0
Cebus capucinus	3	0	34-301	0	0	0	0
Alouatta villosa	2	0	1	0	0	0	0
Saimiri sciureus	1	0	289	0	0	0	0
	Monkey to Monkey						
Aotus trivirgatus	1	0	<1	0	0	0	0
Saguinus geoffroyi	1	0	42	0	0	0	0

+ Demonstrable parasitemia seen for at least three successive days.

TABLE III

SUMMARY OF MOSQUITO FEEDING AND SPOROZOITE TRANSMISSION
FROM OCTOBER 1, 1965 to APRIL 30, 1967

Host	Plasmodium Species	No. of Lots	No. Mosquitoes Fed	No. Mosquitoes Dissected	% Lots Infected	% Infections of Lots Infected
Man	P. falciparum	31	250	19	52	47
Man	P. vivax	5	150	33	40	26
Aotus trivirgatus	P. vivax	158	374	23	36	26
Saguinus geoffroyi	P. falciparum	5	39	19	0	0
Saguinus geoffroyi	P. vivax	27	386	17	7	7
Ateles fusciceps	P. vivax	11	389	16	18	13
Ateles geoffroyi	P. vivax	6	406	17	0	0

Totals

Lots	241
Mosquitoes Applied	93,433
Mosquitoes Fed	84,477
Mosquitoes Dissected	5,072
Lots Infected	80
Percent Lots Infected	33
Average Percent Infected of Lots Infected	30
Transmission of P. falciparum from Man to Primates:	
Attempts	19
Successes	0
Transmission of P. vivax from Primates to Man:	
Attempts	2
Successes	2
Transmission of P. vivax from Primates to Primates:	
Attempts	4
Successes	0

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13. ABSTRACT

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