

SUSCEPTIBILITY OF PANAMANIAN PRIMATES TO
PLASMODIUM VIVAX

BY

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THE desirability of finding a laboratory animal susceptible to *Plasmodium vivax* of man has long been recognized. Aberle (1945) listed many early trials to accomplish this goal. He reported that all trials ended in failure except those with *P. vivax* in the chimpanzee (*Pan Satyrus*) (Mesnil and Roubaud, 1917 and Rodhain and Muylle, 1938). Since that report, unsuccessful efforts were made to infect *Macaca mulatta* with *P. vivax* (Huff and Coulston, 1948; Hawking and Thurston, 1952; Garnham and Bray, 1956; Jeffery, 1961; and Eyles *et al.*, 1961). Huff and Coulston (1948) also reported unsuccessful attempts to infect *Cercopithecus aethiops sabaeus*, *Cercopithecus aethiops pygerythrus*, *Cercocebus fuliginosus*, and *Papio papio* with *P. vivax*. Apparently no efforts have been made to infect New World primates. The present report gives the results of attempts to infect primates of the Western Hemisphere with *P. vivax*.

Materials and Methods

Primates are purchased individually at the laboratory. Daily blood films are made from each animal for the first five weekdays and then weekly thereafter. In addition, blood is collected for sera, blood cell counts, hemoglobin and hematocrit determinations, and the animals are numbered by tattoo and weighed. Fecal examinations for intestinal parasites are made at periodic intervals and, if necessary, animals are treated. Fecal swabs are taken and checked for pathogenic bacteria. Young

spider monkeys are deloused when necessary. Tuberculosis has never been found in primates at the laboratory so no tuberculin testing is done. Usually animals are kept in individual cages during the first week at the laboratory. Later they are placed in larger cages with several others, the number depending on the size and species of primate and the size of the cage. Juvenile and adult primates are fed fruits, vegetables and bread supplemented by vitamins and minerals, varied according to species. Infants are fed baby cereals, fruits, and milk. Dead monkeys are autopsied and tissues saved for further study.

Thus far all primates used were either intact and untreated, splenectomized, treated orally by stomach tube with a 6-mercaptopurine¹ (Purinethol® and Imuran®), and/or injected intramuscularly with a nitrogen mustard compound² (Endoxan®). The initial dose of 6-mercaptopurine was 5.0 mg./Kg. body weight, followed at times by a daily dose of 2.5 mg./Kg. for a few days. When a nitrogen mustard compound was used, it was injected at the same time as the malaria blood at the rate of 10 mg./Kg. body weight.

Vivax bloods were obtained from persons living within 75 miles of the laboratory. Initially the bloods were injected either intravenously or intraperitoneally. Now all bloods are injected intraperitoneally.

Blood films are made at least daily on primates on test. Ordinary thick smears are made until a countable parasitemia develops, then Earle-Perez smears (Earle and Perez, 1932) and thin blood films are made. Parasite density is determined by examining at least 0.1 cmm. of blood on the Earle-Perez film. Positive animals are examined until thirty days after the last positive smear and then at least

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¹ Purinethol or Imuran: Burroughs, Wellcome and Co., Inc., Tuckahoe, N.Y., U.S.A.

² Endoxan-Asta: Asta-Werke Ag, Chemische Fabrik, Brackwede, Germany.

weekly. Smears must be negative for at least thirty days after inoculation of malaria blood before an animal is considered uninfected. Then if the animal is intact, it is splenectomized and examined daily for another thirty days before a final determination of its status is made.

Anopheles albimanus, a mosquito that has been maintained in colony at the laboratory for many years, is used in vector transmission studies. Mosquitos are three days old when fed on infected primates or humans. Before the blood meal they have been allowed only access to cotton pads moistened with water. In attempts to infect the vectors upon small primates, several hundred mosquitos are placed in a cage 18 cm. \times 18 cm. \times 18 cm. The top and one side are nylon netting. The other four sides are cloth. A cloth sleeve attached to one of the cloth sides permits entrance into the cage. The cage is suspended from a wire framework by corner tie strings. The animal is taped to a piece of plywood 45 cm. \times 14 cm. that has the center part measuring 30 cm. \times 8.5 cm. removed. The animal is placed on top of the cage with its shaved ventral surface exposed to the mosquitos. Most mosquitos feed quickly through the nylon netting. Mosquitos not feeding are removed with a suction tube while the feeding proceeds. Later the fed mosquitos are allowed access to cotton pads soaked in 2 per cent syrup. Pads are changed daily. The mosquitos are maintained at $76 \pm 2^\circ\text{F}$. at a high relative humidity. Petri dishes containing moistened gauze pads are kept in the bottom of the cages for the deposition of eggs.

Dissections of midguts from samples of mosquitos are made between the sixth and tenth days, of the midguts and salivary glands between the eleventh and fourteenth days, and of the salivary glands subsequent to the fourteenth day after feeding. The number of oocysts and sporozoites are counted and graded according to the method of Young *et al.* (1946).

Mosquitos used in transmission attempts are placed singly in numbered plastic vials with one end covered with bobinette and the other end covered with two overlapping pieces

of dental dam. Mosquitos are fed by the interrupted feeding technique. The salivary glands of all or a sample of the mosquitos are then dissected to determine sporozoite rate. Thick blood films from human volunteers or primates on which mosquitos have fed are examined starting at the sixth day after feeding.

Results

Attempts made to infect 37 primates representing six different species with *P. vivax* from man are summarized in Table I. No successes were obtained with either four whiteface monkeys, *Cebus capucinus*; eight black spider monkeys, *Ateles fusciceps*; one red spider monkey, *Ateles Geoffroyi*; or two howler monkeys, *Alouatta villosa*. Two of 10 attempts with the titi marmoset, *Saguinus geoffroyi*, were successful. The parasite inoculum used to infect the two marmosets was 29.9×10^6 (Table II), the prepatent period was one day, and the patent period seven days, and the maximum parasitemia only 200 per cmm. Seven of 11 attempts with the night monkeys, *Aotus trivirgatus*, were successful. The parasite inoculum used in the night monkeys ranged from 20.6 to 95.9×10^6 , the prepatent period from 1 to 10 days, the patent period from 10 to 52 days and is continuing in some monkeys, and the maximum parasitemia reached 59,800 per cmm.

From the infected monkeys, attempts were made to infect 27 primates representing five different species (Table I). Eleven of 15 night monkeys, all inoculated with *vivax* bloods from night monkeys were infected. The parasite inoculum ranged from < 0.1 to 8.7×10^6 per cmm., the prepatent period ranged from 1 to 14 days, the patent period up to 58 days and is continuing, and the maximum parasitemia up to 34,940 per cmm. No other primates became infected.

Table II reports data on each primate infected with *P. vivax*. It shows that night monkeys became infected when either intact and untreated, splenectomized, administered 6-mercaptopurine, or both splenectomized and administered 6-mercaptopurine. One of the two marmosets was intact and untreated and the other was administered 6-mercaptopurine.

TABLE I

TRANSFER OF *Plasmodium vivax* IN BLOOD FROM JANUARY 1, 1965 TO APRIL 15, 1966

Species of Monkey	Man to Monkey		Inoculum 10 ⁶	Prepatent Period	Patent Period	Parasitemia Maximum
	Attempts	Successes*	Range	Range Days	Range Days	Range mm ³
<i>Aotus trivirgatus</i>	11	7	20.2-95.9	1-10	10-52**	<10-59,800
<i>Saguinus geoffroyi</i>	10	2	2.0-35.4	1	7	<10-200
<i>Cebus capucinus</i>	4	0	2.0-10.5	—	0	0
<i>Ateles fusciceps</i>	8	0	0.4-95.9	—	0	0
<i>Ateles geoffroyi</i>	1	0	?	—	0	0
<i>Alouatta villosa</i>	2	0	20.2	—	0	0

Monkey to Monkey						
Species of Monkey	Monkey to Monkey		Range	Range Days	Range Days	Parasitemia Maximum
	Attempts	Successes*				
<i>Aotus trivirgatus</i>	15	11	<0.1-8.7	1-14	6-58**	350-34,940**
<i>Saguinus geoffroyi</i>	5	0	<0.1-6.0	—	0	0
<i>Cebus capucinus</i>	3	0	1.4-15.5	—	0	0
<i>Ateles fusciceps</i>	3	0	1.3-44.0	—	0	0
<i>Ateles geoffroyi</i>	1	0	1.4	—	0	0

* Demonstrable parasitemia for at least three successive days.

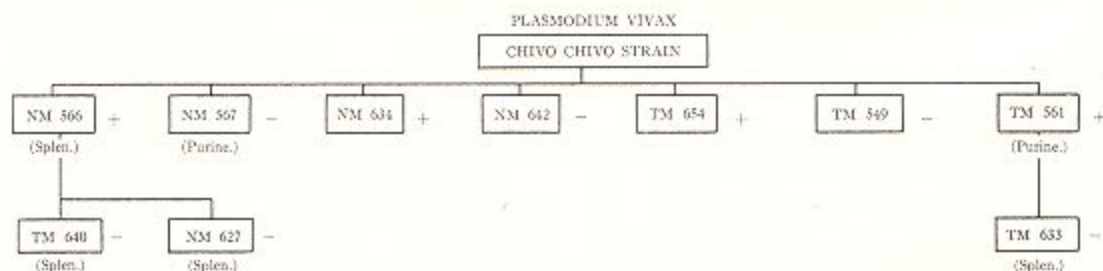
** Parasitemia continuing.

— = No infection.

The first successful transmission of *P. vivax* (Chivo Chivo strain) was obtained using citrated blood containing 5,970 parasites per cmm., from a patient hospitalized in the Panama Canal Zone with the Chivo Chivo strain (Fig. 1). Two of 4 night monkeys and 2 of 3 marmosets became infected. Three attempts to subinoculate the infection to other primates were unsuccessful.

Figure 2 shows the first successful monkey to monkey transmission of *P. vivax*. The

blood containing 9,594 parasites per cmm., was obtained from a person living in Achote near Colon on the Atlantic coast. It was citrated and inoculated into black spider monkey 742 and night monkey 736. The night monkey developed infection. Figure 3 shows the parasitemia exhibited by night monkey 736 after inoculation from the human host. The maximum parasitemia was 15,260 per cmm. The monkey was treated with chloroquine on the 49th day after inoculation but



+ = Demonstrable parasitemia seen for at least three consecutive days.

- = Parasitemia absent or present for less than three days.

NM = *Aotus trivirgatus*.TM = *Saguinus geoffroyi*.

Splen. = Splenectomized.

Purine = Purinethal (a 6-mercaptopurine compound).

Fig. 1. Transmission of Chivo Chivo strain of *Plasmodium vivax* to *Aotus trivirgatus* (NM) and *Saguinus geoffroyi* (TM).

TABLE II

Plasmodium vivax INFECTIONS IN THE NIGHT MONKEY, *Aotus trivirgatus* AND THE TITI MARMOSET, *Saguinus oedipus* TRANSMITTED BY INTRAPERITONEAL INOCULATIONS OF INFECTED BLOOD

No. of Monkey	Species of Monkey	Source of Plasmodium strain	Treatment or Surgery	Inoculum 10 ⁶	Prepatent Period Days	Patent Period Days	Parasitemia Maximum mm ³
634	<i>Aotus</i>	Man	None	29.9	2	12	<10
566	<i>Aotus</i>	Man	Splen.	29.9	1	10*	5,970
740	<i>Aotus</i>	Man	Imuran	20.6	6	36	1,520
761	<i>Aotus</i>	Man	Imuran	20.6	5	34*	59,800
736	<i>Aotus</i>	Man	Imuran and Splen.	95.9	10	49*	15,260
773	<i>Aotus</i>	Man	Imuran and Splen.	56.6	4	37*	24,680
776	<i>Aotus</i>	Man	Imuran and Splen.	56.6	4	52**	47,030**
783	<i>Aotus</i>	<i>Aotus</i>	None	3.0	1	9*	1,760
795	<i>Aotus</i>	<i>Aotus</i>	None	2.1	7	43**	5,590**
788	<i>Aotus</i>	<i>Aotus</i>	Splen.	1.3	3	41*	34,100
789	<i>Aotus</i>	<i>Aotus</i>	Splen.	0.4	6	49**	34,940**
779	<i>Aotus</i>	<i>Aotus</i>	Splen.	0.05	12	24*	13,310
782	<i>Aotus</i>	<i>Aotus</i>	Imuran	1.1	3	58**	1,910**
801	<i>Aotus</i>	<i>Aotus</i>	Imuran	8.7	6	23**	10,330**
755	<i>Aotus</i>	<i>Aotus</i>	Imuran and Splen.	<0.1	5	6*	350
764	<i>Aotus</i>	<i>Aotus</i>	Imuran and Splen.	0.1	9	40*	30,410
784	<i>Aotus</i>	<i>Aotus</i>	Imuran and Splen.	0.2	14	53**	13,370**
785	<i>Aotus</i>	<i>Aotus</i>	Imuran and Splen.	0.3	8	36**	3,730**
654	<i>Saguinus</i>	Man	None	29.9	1	7	<10
561	<i>Saguinus</i>	Man	Purine.	29.9	1	7	200

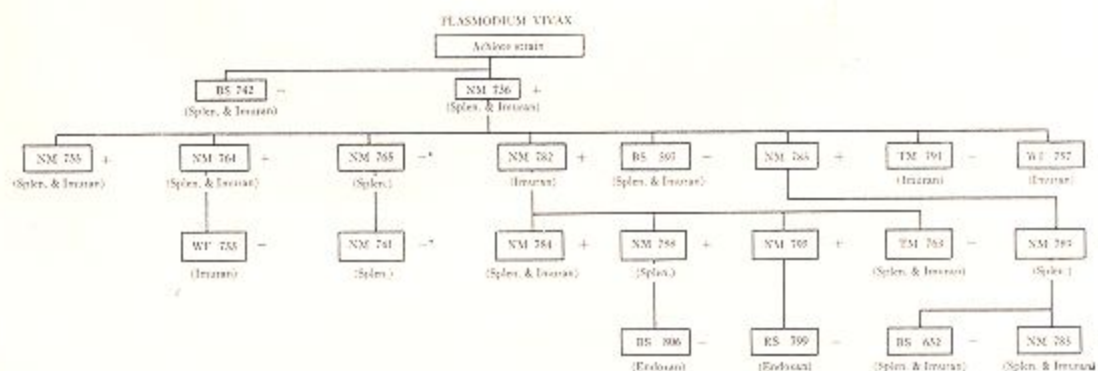
* Died while parasitemia patent.

** Parasitemia continuing.

Splen. = Splenectomized.

Purine. = Purinethol (6-mercaptopurine).

Imuran = 6-mercaptopurine.



+ = Demonstrable parasitemia seen for at least three consecutive days.

* = NM 741 showed 1 parasite after reinoculation from NM 765.

- = Parasitemia absent or present for less than three days.

NM = *Aotus trivirgatus*.

BS = *Aotus fusciceps*.

TM = *Saguinus oedipus*.

WT = *Cebus jacchus*.

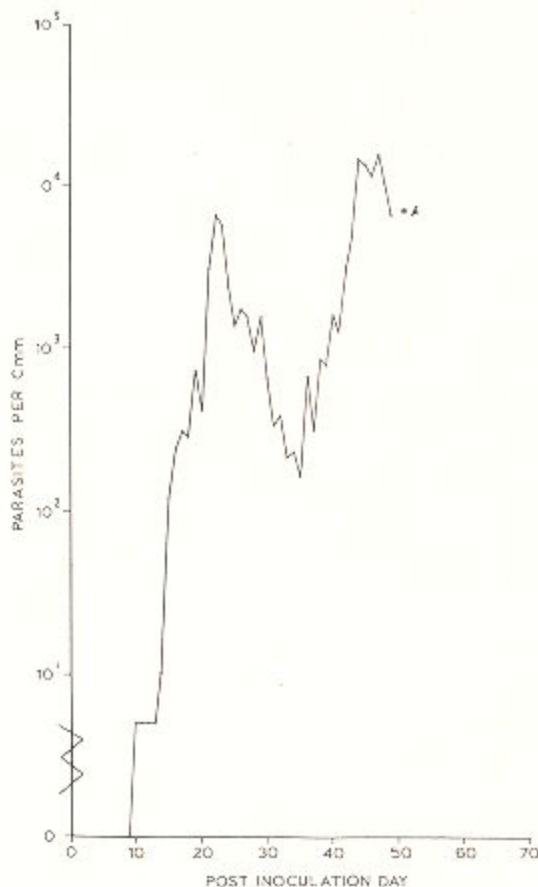
ES = *Aotus geoffroyi*.

Splen. = Splenectomized.

Imuran = 6-mercaptopurine.

Endosan = Nitroquin oxaline.

Fig. 2. Transmission of Achiote strain of *Plasmodium vivax*.



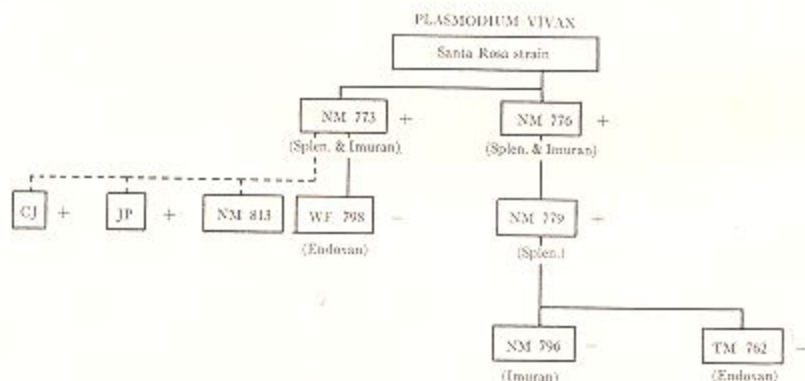
Plasmodium vivax (Achiote strain) parasitemia in Night Monkey #736
 * Chloroquina administered. † Died

Fig. 3. *Plasmodium vivax* (Achiote strain) parasitemia in Night Monkey #736.

died the same day. Serial passages have been made from monkey 736 involving 5 species of primates (Fig. 2). So far the infection has been through three consecutive monkey to monkey passages.

We reported recently the first transmission of *P. vivax* from man to monkey to man (Young *et al.*, 1966). The sequence of events is shown in Figure 4. Both of the night monkeys inoculated intraperitoneally with *P. vivax* blood from the human source became infected. *Anopheles albimanus* mosquitos were fed repeatedly on these monkeys. One group of mosquitos fed on monkey 773 on the 32nd day of patency showed good gut infections and sporozoites in the glands 13 days after feeding. The infections were transmitted to two human volunteers by the bites of these mosquitos but so far (21 days later) the monkey bitten by the same mosquitos has not developed a parasitemia.

Figure 5 shows another example of *P. vivax* transmission from man to monkey to monkey using the Emperador strain. Blood was obtained from a patient hospitalized in the Panama Canal Zone who had acquired the infection in the jungles west of the Panama Canal. The blood, containing 2,060 parasites per cmm., was citrated and inoculated into black spider monkey 652 and into two night mon-



+ = Demonstrable parasitemia seen for at least three consecutive days.
 - = Parasites absent or present for less than three days.
 — = Blood inoculation.
 - - - = Sporozoites by mosquito bite.
 NM = *Notus trivirgatus*.
 WF = *Cebus capurinus*.
 TM = *Sapajous jefferysi*.
 C. J. and J. P. = Human volunteers.
 Splen. = Splenectomy.
 Imuran = 6-mercaptopurine.
 Endosan = Nitrofen mustard.

Fig. 4. Transmission of Santa Rosa strain of *Plasmodium vivax* from man to monkey to man.

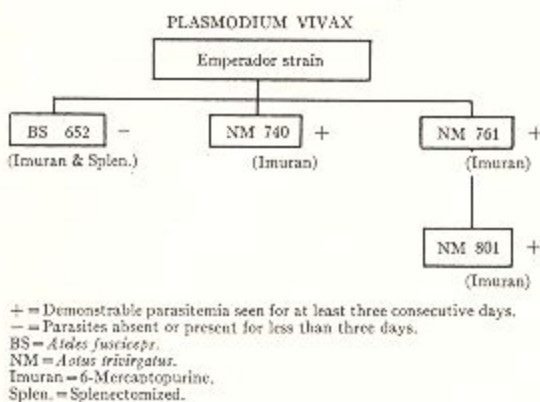


Fig. 5. Transmission of Emperador strain of *Plasmodium vivax*.

keys, numbers 740 and 761. The two night monkeys were infected and the infection was transmitted from one night monkey (761) to another (801). Night monkey 761 reached a peak parasitemia of 59,800 per cmm. on the 32nd post-inoculation day. This is the highest parasitemia of *P. vivax* in primates recorded in our studies so far.

Table III shows the results of vector transmission studies using *Anopheles albimanus*. Thirty-two lots comprising 13,246 mosquitos were fed on night monkeys showing gametocytes of *P. vivax*. The male gametocyte count ranged from <10 to 500 per cmm., while the female count ranged from <10 to 1,000. Of 770 midguts, only 1.9 per cent showed oocysts and, of 331 salivary glands, 23.8 per cent showed sporozoites. However, individual lots showed much higher percentages of infections: i.e. a 57 per cent sporozoite infection rate of the lot of mosquitos that transmitted the infection from monkey to man.

Discussion and Conclusions

Previous to this report, various attempts had been made to infect primates of the Old World with *P. vivax*. Apparently the only animal shown to be susceptible was the chimpanzee.

We have begun testing New World primates for their susceptibilities to human malaria. Of six species native to Panama, two, viz., *Aotus* and *Saguinus*, appear to be susceptible; the latter much less than the former.

Four strains of *P. vivax* malaria, i.e., Santa

Rosa, Achote, Emperador, and Chivo Chivo, were induced successfully by the injection of infected blood. The infection can run a fairly long and intensive primary course. Some monkeys succumb to the infection. The infection can be transmitted from night monkey to night monkey, by blood inoculations. Gametocytes develop and at least one species of mosquito, viz., *Anopheles albimanus* can serve as a vector.

The infections appear in both intact monkeys, in those receiving immunosuppressive drugs and in those which have been splenectomized. The highest infections have appeared in the modified animals but insufficient numbers of intact animals have been used to make a significant comparison.

The night monkey appears to be a promising host for the laboratory study of *P. vivax* malaria. If acquired young, it becomes tame and easy to handle. The adults weight 600 to 800 grams.

Natural malaria infections have never been found in *Aotus* or *Saguinus* primates in Panama or elsewhere (Dunn and Lambrecht, 1963; Porter *et al.*, 1966).

Many aspects of this human malaria-monkey model are being investigated at the Gorgas Memorial Laboratory.

Summary

Six species of New World monkeys have

TABLE III

INFECTIVITY TO *Anopheles albimanus* OF *P. vivax* INFECTIONS OF HUMAN ORIGIN INDUCED IN *Aotus trivirgatus* (NIGHT MONKEYS)

Lots of mosquitos fed	32
% of lots with some mosquitos infected	31.3
No. of mosquitos fed	13,246
Gametocytes/mm ³	
♂ range	<10-500
♀ range	<10-1,000
Temperature	76 ± 2°F.
Dissection results:	
Midguts	770
% positive cocysts	1.9
Salivary glands	331
% positive sporozoites	23.8
Transmission attempts to man	2
% successes	2

been tested for their susceptibility to *Plasmodium vivax* of human origin. Infections resulted in two species, *Saguinus Geoffroyi* (tit marmosets) and *Aotus trivirgatus* (night monkeys), the latter developing high parasitemias. The infections were transmitted to other night monkeys by blood inoculations. *Anopheles albimanus* mosquitoes were infected by feeding upon malarious monkeys and shown to be vectors.

Acknowledgments

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