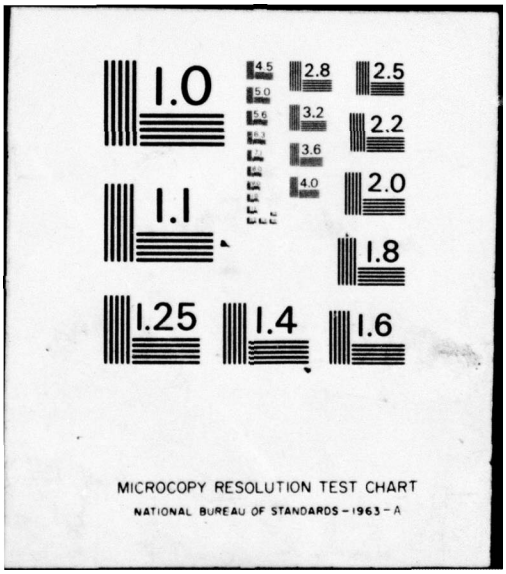
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MONKEYS AS HOSTS OF HUMAN MALARIA AND OTHER PATHOGENS

FINAL REPORT

Richard N. Rossan*

David C. Baerg

August, 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D. C. 20314

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Balboa Heights, Canal Zone

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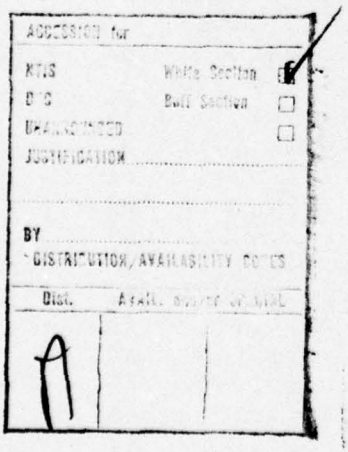
* Co-investigator, replaced Dr. Martin D. Young as Principal Investigator, March 30, 1974.

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A passage line of the Panama II strain of Plasmodium falciparum was produced in Alouatta villosa trabeata and viability of the Vietnam-Oak Knoll was confirmed after 3 years of cryopreservation. Also for the first time, intact Ateles fusciceps and A. geoffroyi were infected with Achiote P. vivax, including Panamanian and Guatemalan subspecies of the latter Ateles. Plasmodium simium developed in unaltered subjects of all 7 species of Panamanian monkeys, 5 of these new experimental hosts. Ten and 28% of monkeys surveyed

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evidenced Trypanosoma (4 species) and Dipetalonema (3 species). Colony mating and parturition was recorded in Cebus capucinus, Saguinus geoffroyi, A. fusciceps, and A. geoffroyi, with a total of 24 viable births. Data were acquired on the description, life cycle and rearing of poorly known jungle mosquitoes, Anopheles neivai, Chagasia bathana and A. squamifemur. Anopheles albimanus malaria infections were best obtained from sporozoite induced parasitemias of Achiote vivax in splenectomized Aotus trivirgatus. Transmission was effected to A. trivirgatus, S. geoffroyi, Saimiri scuireus and both Ateles spp.; exoerythrocytic stages were demonstrated in each of these systems and C. capucinus and C. apella, refractory to the blood phase.

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SUMMARY

This is the final report for the contract supporting 5 years of basic, specialized, and ancillary investigations of induced human malaria in New World monkeys. The ultimate goals achieved were the establishment of models as substitutes for human volunteers.

Two strains of Plasmodium falciparum were acquired and studied in detail. The indigenous Panama II strain was infective for Aotus trivirgatus, Saimiri scuireus, and Saguinus geoffroyi; serial passage was demonstrated in the first 2 species. The first falciparum passage line was achieved in Alouatta villosa hosts, using a subspecies from western Panama. The second chloroquine resistant strain (Vietnam- Oak Knoll) produced fulminating infections in Aotus and moderate parasitemias in Saimiri. Viability of the parasites was confirmed after a 3-year period of cryopreservation.

Some 206 Aotus were inoculated by trophozoites of the Achiote strain of P. vivax, with infections occurring in 95 per cent. Using this strain, intact Ateles fusciceps and A. geoffroyi were infected with vivax malaria for the first time, including Panamanian and Guatemalan forms of the latter Ateles. The virulent Vietnam-Palo Alto vivax in Aotus gave parasite densities at least 2-fold greater than the mean maximum values normally seen for New World strains. A third isolate, Rio Meta, was the first P. vivax from Colombia to be established in monkeys and was especially infective for Saguinus.

Infections of P. simium, the tertian monkey plasmodium occurring only in Brazil, developed in unaltered subjects of all 7 kinds of Panamanian monkeys, 5 of them representing new experimental systems. Among the new hosts, Saguinus was the most receptive. The development of P. simium in Cebus capucinus and Alouatta was particularly interesting in that they are refractory to the blood phase of the human counterpart, P. vivax.

Naturally occurring P. brasilianum and a strain adapted to Aotus monkeys additionally were tested.

The incidence and identification of non-malarial blood parasites in monkeys were determined. Ten and 28 per cent of the animals surveyed evidenced Trypanosoma (4 species) and Dipetalonema (3 species), respectively; each of the 7 species of monkeys demonstrated infection of both types of parasites, largely dependent upon geographical origin.

Mating and parturition were recorded in C. capucinus (2 native subspecies), S. geoffroyi, A. fusciceps, and A. geoffroyi (2 subspecies). A total of 24 viable births occurred.

Larval and adult mosquito material were obtained routinely from a diverse spectrum of habitats. The jungle study areas (Altos de Pacora and Cerro Nique) were especially utilized for collection and dissection of arboreal anophelines, ecologically associated in Panama with natural transmission cycles of blood parasites in monkeys. Canopy collections yielded Anopheles neivai and Chagasia bathana. Vector stages of malaria, trypanosomes or filariae were not found during this study, while it was determined that parous females comprised approximately half of the biting population and that the parasites were present in monkeys concurrently examined.

Anopheline colony maintenance included A. albimanus, the standard line and a newly-colonized strain from Panama, and A. aztecus. Seven other species demonstrated cage mating or were used in induced copulation studies to produce laboratory populations. Base line data were acquired on the description, life cycle and rearing of several poorly known jungle anophelines, viz., A. neivai, C. bathana, and A. squamifemur.

More than 5,000 pools of Anopheles were fed in tests on monkeys bearing human or simian malaria infections. While the monkey malarias and P. falciparum were only poorly or non-infective for the mosquitoes, the efficacy of P. vivax was demonstrated. For P. vivax in Aotus, a greater consistency of positive feedings was obtained from sporozoite induced infections in splenectomized hosts, although individual monkey variability could be a greater factor in determining gametocyte infectivity than the type of induced infection. During the inclusive trials, the Achiote strain proved to be vector infective from 4 of 5 experimental host species. The newly colonized A. albimanus (Escobal) was not significantly different than the standard GML strain (maintained in the insectary for more than 35 years) for vivax susceptibility. Companion feedings of A. albimanus against A. aztecus gave equivocal results. Other species of mosquitoes infected from monkeys included A. triannulatus, comparable to those seen with A. albimanus. Concommitant dissections of fed mosquitoes to determine the presence of vector stages of other blood parasites of monkeys were negative, including tests with Culex spp. and phlebotomine sand flies.

All sporozoite induced infections were effected via A. albimanus with the Achiote strain of P. vivax, and were achieved in each of 5 species of monkeys proven susceptible to the

trophozoite stages. As few as 4 mosquitoes infected monkeys. In transmission trials with 529 inoculated monkeys, all A. geoffroyi became infected while the poorest frequency was demonstrated by Aotus. Ratios of positive to total animals were appreciably higher in splenectomized than in intact subjects only among the Saimiri and Saguinus. Ateles (both species) were significantly more susceptible to sporozoite-induced infections than co-recipient Aotus. Studies with DL-methionine administered to recipients showed an apparent advantage for parasite development in the P. vivax-A. geoffroyi system, as prepatent periods were 3 to 8 times longer in the untreated controls.

As part of the total evaluation of monkeys as models for human malaria, extensive efforts were directed toward the study of vivax exoerythrocytic (EE) stages. Eight species of nonhuman primates were utilized, and the tissue forms were demonstrated from 7 (6 of these systems for the first time). In Panamanian A. geoffroyi, 7 and 9-day EE stages were seen; those from an animal administered DL-methionine were significantly larger at 7 days than in the control subject. Seven, 9, and 10-day bodies were demonstrated for A. fusciceps and Saimiri. In the latter host unusual forms were encountered, distinguished by the presence of several large, opaque, rounded aggregates within the bodies. A single EE schizont, suggesting arrested growth, was found in Saguinus from a biopsy at 7 days. Both C. capucinus and C. apella supported EE development at 7, 9, and 10 days. Although Cebus is refractory to the blood phase, the tissue schizonts exhibited typical growth similar to that seen in recipients of other host species that did develop patent infections. In other trials, findings with Aotus (one body at 11 days) confirmed the work of others, while no EE stages were evidenced in Alouatta hosts.

The availability of various biological material during this program afforded an unique opportunity for cooperative projects; investigators were represented at GML, Panama, Canal Zone, and some 15 laboratories outside this country.

FOREWORD

In conducting the research described in this report, the investigators 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.

INTRODUCTION

This is the final report for the contract initiated on 1 September 1971 and terminated 31 December 1975. The basic investigations on the susceptibility of New World monkeys to human plasmodia were first begun under previous Army Research and Development grants and contracts. Studies supported by this succeeding contract extended and diversified the preceeding programs.

We have shown that the 7 species of Panamanian monkeys possess varying degrees of susceptibility to the two most important plasmodia of man, Plasmodium vivax and P. falciparum. Other phases of our work have included obtaining information on naturally-occurring blood parasites of these monkeys. The feasibility of establishing breeding colonies of certain Panamanian species of primates, animal management and husbandry were part of the above studies. Cooperative projects were initiated and fostered throughout the contract period in order to obtain the maximum utilization of biological material.

The goals achieved were the assessment and characterization of relationships of the host, vector and malarial parasites, with the establishment of models as substitutes for human volunteers in drug testing and other investigations.

PROJECTS

I. Development and Assessment of Monkeys as Models

A. Human Plasmodia

1. P. falciparum

Two strains were acquired and studied in detail. Both were resistant to chloroquine and had been adapted to Colombian Aotus trivirgatus prior to receipt at this laboratory. The first, Panama II, was obtained at the second monkey transfer from Dr. W. Collins, NIH, Chamblee, Georgia. Inasmuch as this represented the only Panamanian falciparum adapted to monkeys, its development was investigated in co-indigenous systems (Tables 1 and 2). At Gorgas Memorial Laboratory (GML), splenctomized Aotus of Colombian origin, also were inoculated initially with the Panama II line to assist in adaptation. These subjects were highly susceptible, but did not succumb to induced infection. When first tried in

Panamanian Aotus, the parasites could be transferred only among splenectomized recipients, however once acclimated, the parasitemias approximated or exceeded those in Colombian Aotus. This line then proved to be highly lethal to splenectomized Panamanian Aotus, as 98% (18 of 21) died during patency. Adaptation of Panama II falciparum to normal Aotus was more difficult than to altered subjects (Table 1). Upon adaptation through serial transfer, however, the primary attack reached more than 1.5×10^6 per cmm. The same number of normal Aotus (19) died during patency as did those that were splenectomized. Infections recrudesced in all 9 normal monkeys that survived the first attack. The patent periods of recrudescences were generally longer, and the counts were higher in 7 of 9 hosts. Lethality was increased during recrudescence as 6 of 9 monkeys died with demonstrable parasitemias. Subinoculation, during these periods, to unaltered recipients did not produce initial heavy parasitemias. The phenomenon of a low parasitemia in the primary attack and a high secondary parasitemia is an interesting one, especially from an immunological view, as normally the primary attack is the more severe episode.

At the 18th Aotus transfer, the Panama II isolate was found to be infective for Alouatta villosa trabeata, the red howler monkey (Table 2). This subspecies, from Chiriqui in western Panama, shows good acclimation to captive conditions in contrast to our experience with the black howler monkey, A. v. aequatorialis, from central and eastern Panama. Of the initial 2 red howler recipients, one experienced an infection, while the other remained negative throughout 54 days of observation. The first animal showed a 1-day prepatent period, having received 738×10^6 parasites. Four subsequent serial passages were achieved. The onset of patency among these subjects, requiring 5-20 days, did not relate to the inoculum size (0.8×10^6 to 70×10^6 parasites). Following the initial Alouatta passage, moderate densities were seen in the primary attack. Peak counts reaching 35,000 per cmm occurred by the first to third week. All 4 animals surviving the initial patent period experienced 2 to 4 recrudescences, with parasites last evidenced 85 to 175 days after introduction. Intensities of these recurrences were variable although persisting for more than 6 weeks in 2 monkeys.

Additional susceptibility trials with Panama II falciparum were summarized in our 1973 Annual Report. Infections developed in all of 4 splenectomized ($>180,000$ parasites per cmm) and 2 of 4 normal (<10 per cmm) Saimiri sciureus and 2 of 4 normal Saguinus geoffroyi. In the former, the initial heavy infections were followed by recrudescences; serial passage was achieved. In four trials using Ateles fusciceps and Cebus capucinus, no

patent infections were detected over long observation periods.

The second chloroquine resistant strain, Vietnam-Oak Knoll (FVO), at acquisition from Dr. W. Siddiqui, University of Hawaii, had been well-adapted to South American Aotus. It was infective to all unaltered Panamanian Aotus inoculated, and fulminating infections were produced such that 70% of the recipients died during patency (Table 3). Among 3 of 4 Saimiri, moderate infections occurred and the animals survived. None of 3 Cebus, 2 Saguinus, or 2 Ateles developed a patent parasitemia during examination of more than 100 days.

Both of the above Aotus-adapted strains were cryopreserved (-70°C) and successfully re-established on an ad hoc basis. The Panama II isolate was viable for Aotus after 300 days and the FVO strain for periods up to 3 years (23-day prepatent period). A third strain, Malayan-Camp, had been cryopreserved for almost 2 years, at which time it was infective for Aotus (prepatent period 17 days), but not for a companion C. capucinus. The latter species has been shown to be susceptible only to the Uganda-Palo Alto strain; trials using 3 additional Aotus-adapted strains, Vietnam-Oak Knoll, Malayan Camp and Panama II, have been negative.

Limited attempts were made to adapt Panamanian falciparum to indigenous Aotus. Parasites from Garachine, Darien were inoculated into 2 recipients (one normal and one splenectomized), but no patent infection developed in either monkey. An inoculum from Turbo, Colombia also proved to be non-infective for Panamanian Aotus during 164 days of observation. Two Cebus recipients received sporozoites via hepatic inoculation derived directly from man and results will be discussed in the vector-associated section.

2. P. vivax

The Achiote strain was maintained by trophozoite passage in Aotus primarily to serve as sources for mosquito infections and inocula for other monkey susceptibility trials. Some 206 Aotus were inoculated during the period of this report, with infections occuring in approximately 95% of the animals. Trophozoite transfer of this strain among primates has been continued since its isolation in 1966. While 5 of the 7 species of Panamanian monkeys were previously shown by us to support parasite development at levels necessary to sustain passage lines, the Ateles species had always required splenectomy. During the present series of investigations intact A. fusciceps and A. geoffroyi yielded demonstrable parasitemias. In each instance, recrudescences occurred, whereas in

splenectomized Ateles single attacks are characteristic of the induced infections. An intact A. geoffroyi, subspecies vellerosus Gray of Guatemalan origin, also was found to be susceptible to Achiote vivax, yielding an infection similar to that with the Panamanian A. geoffroyi panamensis Kellogg and Goldman. The Guatemalan monkey can be separated readily from the subspecies indigenous to Panama by its distinctive pelage coloration.

A vivax strain from Rio Meta, Colombia was established in an unaltered Aotus by blood subinoculation from man; a splenectomized Saimiri also was infected in this initial passage. Such an isolation afforded the initial opportunity to study a non-indigenous vivax parasite in Panamanian monkeys, and represented the first of Colombian origin adapted to monkeys. The infections in Aotus were comparable to those obtained with Achiote vivax (Panamanian), while parasitemias in splenectomized Saguinus were greater than 70,000 per cmm (vs. 35,000 per cmm in intact Saguinus). After 6 serial passages in monkeys, the work with the Rio Meta strain at GML was terminated and the parasites cryopreserved.

The Vietnam-Palo Alto strain of P. vivax, which had been adapted to Colombian Aotus, was received from Dr. A. Voller. This highly virulent strain retained such characteristics in Panamanian Aotus, as summarized in the Annual Report of 1973-1974. The infection recrudesced in a single survivor among a total of 7 hosts. After the first passage the average maximum parasitemia reached concentrations higher than 10^5 per cmm. Such parasite densities are at least 2-fold greater than the mean maximum values normally seen for the New World P. vivax. Saguinus was moderately infected (27,730 parasites per cmm) while Saimiri was poorly susceptible (< 10 per cmm). Alouatta was refractory to the blood stages during examination periods up to 112 days.

3. P. malariae

Attempts were made to establish a monkey-adapted West African strain in Panamanian Aotus, with a frozen sample obtained from Dr. Voller. Two splenectomized recipient monkeys were examined over periods of 23 and 65 days; a blind passage was made to a third splenectomized Aotus from the latter animal, and was followed for 110 days. Patent infections were not demonstrated. A second isolate, from a newly-discovered focus in Panama (Don Bosco) was tested in 3 intact Aotus subjects. No parasitemias were recorded over periods of 53 to 140 days.

Due to the inability to achieve a passage line of P. malariae at this laboratory, 5 Aotus were shipped to Dr. Voller in order to facilitate adaptation with frozen samples there. No positive results followed, due either to the loss of parasite viability or insusceptibility of Panamanian Aotus.

B. Simian Plasmodia

1. P. simium

This tertian monkey parasite has been found only in circumscribed areas of Brazil. A strain was obtained through the courtesy of Dr. R. Nussenzweig, New York University, and a parasite line then was established in Panamanian Aotus (Table 4). P. simium was highly invasive, as infections showed in 18 of 21 recipients. Seventeen subjects succumbed during patency (between the 32nd and 87th day), in most cases with fulminating parasitemias of more than 100,000 per cmm. Deaths in more than 2/3 of the cases occurred during the descending phase of the primary attack. Heavy infections were produced in 2 additional Aotus incoulated with blood samples that had been preserved at low temperature (-70°C) for 80 days.

The Panamanian Saimiri were shown to support only a low grade primary attack, in contrast to the high parasitemias recorded by workers using South American Saimiri. Only one monkey (6960) experienced significantly higher parasite development during recrudescence than in the primary infection.

Data for the 5 other species of Panamanian monkeys tested, all representing new host systems for P. simium are detailed in Table 5. Of these, S. geoffroyi was the most receptive, showing parasitemias comparable to those in Aotus. The black howler monkey, A. villosa, experienced a moderate infection and died 8 days after 23 days of patency. Prepatent periods were longest in C. capucinus and the 2 positive animals were mildly infected; trophozoites did not reappear for more than 100 days after blood smears became negative. The refractory Cebus (4896), the only splenectomized animal in this study, was examined for 52 days post inoculation. The primary attack in A. fusciceps and A. geoffroyi showed only marginal parasitemias, persisting over a 3-week period. A recrudescence occurred in each of these Ateles, producing even lower parasitemias with shorter patent periods. After termination of the second attack, the animals remained negative for more than 200 days.

An interesting observation is that P. vivax, the human counterpart of P. simium, cannot be maintained in Cebus and Alouatta. The development of P. simium in these models therefore would appear to further differentiate P. simium and P. vivax.

2. P. brasilianum

Chronic infections, induced by trophozoites of indigenous strains of this quartan malaria, were demonstrated in splenectomized Ateles. Occasional high parasitemias (500,000 per cmm) followed, requiring chemotherapeutic intervention at a sub-curative dose.

An Aotus-adapted inoculum of P. brasilianum (exact Neotropical origin unknown) was acquired from Dr. A. Voller, Nuffield Institute of Medical Research, London. In our studies Panamanian Aotus were highly susceptible,with parasitemias of more than 100,000 per cmm and substantial gametocytemias (Table 6). Splenectomized C. capucinus also had heavy infections, while Saguinus was moderately susceptible and Saimiri was virtually refractory. Aotus and Saguinus (except only in one instance) are not within the known natural host range for P. brasilianum.

The major emphasis of P. brasilianum concerned the field and vector aspects. For these purposes Panamanian isolates, from Cerro Nique, Cerro Azul, and Rio Chico, were obtained in addition to the above. Experimental vector results will be discussed in the appropriate section.

C. Non-malarial Blood Parasites

Part of the following data were obtained in a cooperative project with the Parasitology Department of Gorgas Memorial Laboratory.

1. Trypanosomes

Approximately 10% of 3,523 animals surveyed either by direct blood examination and hemoculture were infected with trypanosomes. Using the former method only, 4.5% of 6,298 monkeys exhibited trypanosomes. Four species of parasites have been identified: Trypanosoma cruzi, T. rangeli, T. minasense, and T. mycetae. T. cruzi, causing a serious disease in man, was demonstrated in Saguinus (12.2%), Cebus (5.0%), A. fusciceps (1.2%) and Saimiri

(1.7%). Saimiri sciureus from western Panama, (S. oerstedii of some authors), was found for the first time to harbor naturally acquired T. cruzi infections in that region (Table 7). T. rangeli, a less serious pathogen of man, was obtained from Saguinus (55.8%) and Cebus (12.5%). T. minasense was evidenced in Saguinus (36.5%), Cebus (10%) and A. fusciceps (2.3%), while T. mycetae was isolated only from Alouatta (1.7%). Of the Aotus examined, 2.3% harbored an unidentified trypanosome; the trypomastigotes, although rangeli-like, differed from both T. rangeli and T. minasense. In 34 A. geoffroyi, studied by blood films only, 5.8% harbored trypanosome (species undetermined). The most heavily infected host species, S. geoffroyi, was collected from localities in 2 provinces, Panama and Colon (Table 8).

2. Microfilariae

The prevalence rate of Dipetalonema was 28% for 6,298 monkeys examined. Three members of this parasite genus were found: D. marmosetae, D. gracile and D. obtusa. D. marmosetae were detected in S. geoffroyi (73.0%), S. sciureus (8.3%), C. capucinus (5.0%), A. villosa (3.4%), A. trivirgatus (2.6%), and A. fusciceps (2.3%). The second species, D. gracile, was recorded only in Aotus (23.0%), whereas the third, D. obtusa, infected C. capucinus (22.5%) and S. sciureus (1.7%). No identifications of the microfilariae were made in A. geoffroyi (8.8% positive).

The overall percentage of mixed infections of microfilariae and trypanosomes was 2.9%, ranging from 0.1% in Aotus to 10.5% in Saguinus. Geographical differences in incidence and host range were apparent. In the Chiriqui Province, 2 of the filariae, D. marmosetae and to a lesser extent D. obtusa , were present in the Saimiri (Table 7). For the central Provinces of Panama and Colon, only one species, D. marmosetae, was demonstrated in the most heavily infected primate, S. geoffroyi (Table 8).

The high microfilaremias, occurring in some A. trivirgatus, did not appear to influence induced parasitemias of falciparum malaria in these subjects.

D. Reproduction in Monkey Colonies

Although specific breeding facilities were not maintained during the malarial investigations, certain animal groups were housed where feasible, as separate entities to encourage repro-

duction. Mating and parturition was recorded in 4 species, viz., C. capucinus, S. geoffroyi, A. fusciceps and A. geoffroyi.

The most consistent breeding occurred in C. capucinus, held in outdoor gang cages at the G.M.L. laboratory compound, Panama City. A complete list of births is given in Table 9; 11 of 23 (48%) occurred prior to the initiation of the contract date. Two troops of C. capucinus imitator, a western geographical variant of C. c. capucinus, were captured in Chiriqui Province and were retained separately. Group 1 was acquired in March, 1970 and was comprised of 6 males and 6 females (adults and subadults). They were confined together for 1-1/2 years. The second troop, consisting of 5 adult and 1 juvenile males with 2 adult and 1 juvenile females, was obtained in July, 1970. All were placed in another gang cage for 1 year (group 2). Each social unit was reduced thereafter in size by occasional removal of individuals through June, 1975. As indicated, during the inclusive 5-year period at least 5 colony female C. c. imitator conceived and produced full term offspring. A total of 10 progeny resulted plus 1 infant from a feral pairing; the latter birth occurred 1 month after acquisition. The first progeny resulting from cage mating were realized 14 and 11 months after arrival of the 2 respective family groups. Three mothers were multiparous, with 4 interbirth intervals ranging from 15 to 27 months ($\bar{x}$=19).

Cebus c. capucinus, from scattered localities east of the Canal Zone, were acquired on an individual basis beginning October, 1965. Males and females (adults and juveniles) were randomly pooled, after acclimation, into units of less than 10 animals. In subsequent holding, 2 to 4 cages of these monkeys were maintained, with ad hoc removal and reintroduction of subjects. Captive pairings in the above, to mid-year 1975, gave a total of 9 viable births. One individual (No. 684), over 3-1/2 years, was identified as having 3 live births. She died during her fourth parturition, 11 months after the third. Although births of the 2 subspecies of Cebus occurred virtually throughout the year, the fewest (2 of 19 with known dates) were evidenced from August to November, corresponding to the season of heaviest rainfall in Panama. Infant mortality was negligible as abandonment was rare during the 3 month period of close post partum maternal association. While all progeny have remained with their family groups, thus far only one second generation pregnancy (in a 5-year old C. c. capucinus) has been noted which ended in a stillbirth at approximately 5 months.

An offspring of A. fusciceps robustus, Panamanian origin, resulted from a 2-year unit, which consisted of one male and 3 females. On the sixth day after birth, the infant was abandoned and could not be recovered promptly for nursery-rearing. In a shipment of 18 monkeys from the New England Regional Primate Center, a group of A. f. robustus of Colombian origin was represented. Within a 2-year period, 2 aborted fetuses (both early and late) resulted. A colony troop of Guatemalan A. geoffroyi vellerosus received at the same time yielded one live birth 2-1/2 years after acquisition; this infant (female), was successfully hand reared. The Ateles species, as Cebus, have exhibited long term survival, as long as 20 years at GML.

The S. geoffroyi were separated into pairs in cages measuring 4x4x2 feet. In most instances, these sets were not maintained for significant periods (>1 year). Two mating pairs, however, did yield twins and a single infant, respectively, and the offspring were healthy and well-accepted by the parents.

Aotus mating, also in small cages,occurred in a 2-year pair; abortion resulted following administration of quinine for control of a high P. falciparum parasitemia.

II. Vector Associated Investigations

A. Field

Larval and adult mosquito material were obtained routinely from a diverse spectrum of habitats, including mangrove swamp on the Atlantic side of the Isthmus, the Chagres River which supplies the Panama Canal, and localities comprising coastal and interior temporary breeding sources. Jungle study areas also were utilized during special studies for collection and dissection of 2 species of arboreal anophelines, Anopheles (Kerteszia) neivai and Chagasia bathana. These mosquitoes, ecologically associated in Panama with natural transmission cycles of blood parasites in monkeys, were considered primary candidates to serve as effective laboratory vectors. Two principal mountain field stations were involved, Altos de Pacora (2,600 feet) of the Panama Province and Cerro Nique (2,000-4,000 feet) of the Darien Province. At Altos de Pacora, a cloud forest transition zone, bromeliads (the sites of A. neivai breeding) grew at ground level and canopy. During late wet season, when entrance by vehicle was possible, a concerted effort was made to determine A. neivai larval incidence in these sources. A total of 24 collecting trips yielded 0-71 larvae per bromeliad (both naturally occurring and sentinel plants, 6-50 sampled per trip), and gave a total of 1,200 immatures. In the

Darien region, representing tropical humid forest (a transition zone between the subtropical and lower montane belts), bromeliads were difficult to harvest; they were restricted primarily to large canopy colonies of plants (often 100 or more) in individual very tall trees. Surveys of forest streams, the habitat of C. bathana, produced significant populations only in the Darien. Adult mosquitoes were captured during these periods attracted by human bait, on tree platforms 40 to 50 feet above ground level (Table 10); this method was more efficient than using animal bait, light traps, and non-arboreal placement. Altos de Pacora yielded only A. neivai, whereas C. bathana additionally was obtained in the Cerro Nique field camp. An interesting observation was that C. bathana females were taken often more than a mile from known breeding sources. Dissections were performed for malaria and other parasite stages and age grading. In the latter case, parous females (having a verified egg laying, and thus blood feeding history) comprised approximately half the biting population during both lows and peaks of abundance. While malaria (P. brasilianum), filaria (Dipetalonema spp.) and trypanosome (Trypanosoma spp.) infections were present in feral monkeys examined concurrently from the Darien study area (Table 11), no malarial infections were acquired by sentinel Ateles and vector stages were not evidenced in the mosquitoes; we had found sporozoites in A. neivai from this locale under prior Army R and D support. Logistical considerations and changes in the objectives during the Contract necessitated termination of these follow-up field vector investigations.

B. Laboratory

A summary of vector species studied is given in Table 12. The standard GML colony of A. albimanus was maintained with routine high pupal yields (approximately 10,000 per day). A microsporidian infection (Nosema algerae), which appeared in this culture, was controlled by egg rinsing procedures. A second strain of albimanus, obtained from a village in Panama (Escobal) with epidemic falciparum malaria, also was colonized to serve in comparative work. A Mexican anopheline, aztecus, was acquired from the parent colony and was sustained continously for routine comparisons. A. pseudopunctipennis was colonized under a previous Army contract and later discontinued; due to the extensive procedures required for re-establishment (largely a result of restrictive mating conditions), no self-sustained cultures of this species were indicated during the current study.

Laboratory adaptation trials with other species yielded several overlapping generations with the river mosquito, A. triannulatus, and the jungle inhabiting A. oswaldoi; cage mating also was seen with A. punctimacula. In addition to modification of caging and ambient cycles, induced copulation was tried for applicability to those mosquitoes recalcitrant to mate in captivity. While limited sperm transfer was achieved by forced mating of A. aquasalis, A. eiseni, and A. neivai (e.g., a single viable egg batch resulted in attempts with 65 males and 80 females of A. neivai), this method was practical with C. bathana and enabled successive laboratory generations. Only A. albimanus and A. aztecus, which possess relatively simple male genitalia could, in our experience, serve equally for induced copulation application (as shown by spermathecal dissection).

An insectary culture of Culex pipiens quinquefasciatus was initiated, altered to mammalian feeding, and continued for ancillary investigations. Specimens of C. aikenii and a phlebotomine, Lutzomyia sanguinaria, were obtained when necessary from other GML Departmental colonies.

Base line data were acquired on the description, life cycle, and rearing of several of the poorly known Anopheles species. Some of these observations follow. A. neivai were found not to produce eggs until usually the second or third complete blood meal, irrespective of mating or ambient parameters, and some eggs were resorbed. Oviposition followed 3-4 days after feeding, with hatch in a succeeding 4-6 days. Survival of insectary larval populations of A. neivai approximated 50%, with growth requiring 20-30 days at room temperature (23°C). The ova of A. neivai were described for the first time. C. bathana was studied and the egg stage, also previously unknown, was detailed. In laboratory populations, 80 percent of the C. bathana females developed ova after the second engorgement, with Christophers' Stage V achieved in 4-5 days; resorption was not uncommon in females resisting oviposition. Larval mortality prior to the third instar often was greater than 50%, with essentially 100% viability observed thereafter. Completion of the aquatic stages required 17-31 days, showing peak pupal yields during the fourth week. A. squamifemur, previously known only from the adult stage, was collected as blood engorged females via horse traps. Oviposition of this deep forest species was induced, and rearing techniques were established to provide material for description and illustration of the egg, larval, and pupal stages. Embryonic development occurred in 4 days and, among the broods reared, the aquatic cycle was completed within 23-26 days. To date the larvae of A. squamifemur have not been found in the field, however a favorable rearing medium was found to necessitate quantities of

organic material - comparable to that described for breeding habitats of other members of the same subgenus, Lophopodomyia.

The survival and reproductive potentials of A. triannulatus and A. aquasalis were determined in conjunction with their suitability as experimental vectors. In laboratory life span replicates, similar results were obtained for both species. A 50% survival of both males and females continued through the second week from emergence, with longevity for males to the seventh week and for females to the sixth week. In comparison to these findings for A. aquasalis, field studies by other workers have demonstrated a relatively brief average life span (signified by disproportinately large numbers of nullipars, or young females). It has been thus reasoned that the ability of A. aquasalis to transmit malaria is limited, unless present in abundance.

C. Malaria Infectivity/Mosquito Susceptibility

During the course of investigations, more than 5,000 Anopheles lots (a pool of 200 to 400 female mosquitoes each) were fed on monkeys bearing human or simian malaria infections (Tables 13 thru 19). Aliquots of 20 or more specimens were dissected per lot (midguts and salivary glands) to determine infections and a total of approximately 125,000 fed mosquitoes were subsequently examined. Efforts were directed toward enhancing and determining gametocyte production and infectivity (e.g., splenectomy, administration of immunosuppressants, influence of host species and parasite strain, degree of adaptation, diel rhythm effects) and mosquito susceptibility (e.g., species testing, post prandial incubation factors). The modifications were largely unproductive for those host parasite combinations which otherwise did not yield vector infections.

With the receipt of Panama II and Vietnam-Oak Knoll falciparum strains, their potential infectivity to indigenous mosquitoes was specifically investigated (Table 13). The Panama II line produced, in some Aotus subjects, unusually high sustained gametocytemias reaching 16,740 per cmm. Up to 42 lots of A. albimanus or A. aztecus were applied to individual hosts, but without the appearance of oocyst development. The Vietnam-Oak Knoll strain in all cases produced only poor gametocytemias in monkeys.

In ancillary investigations supported by GML, using natural infections, positive yields (up to 52%) occurred in A. albimanus with the indigenous P. falciparum, Guayabalito-4; a Colombian strain gave poor infections in this test vector, apparently due

to the prior chemotherapy. A low grade infection of P. malariae was non-infective for A. albimanus in one instance tried.

Plasmodium vivax testing comprised the majority of vector feedings (3,514 lots), using primarily the Achiote strain (Table 14) which has been adapted to Aotus since 1965. Anopheles albimanus infections from 40 Aotus in trophozoite passage lines were compared with vector infectivity of 19 sporozoite induced vivax parasitemias for this host species, comprising both intact and splenectomized subjects. The sprozoite induced infections were found to be more infective than those induced by trophozoites in intact animals (24 vs 13% positive lots), however in both types of sources better respective donor infectivity was recorded among the splenectomized animals (38 vs 18% positive lots). There was a wide range of male gametocyte concentrations (<10 to 4,000 per cmm), with 500 per cmm recorded in 17 of 26 splenectomized in contrast to only 8 of 33 intact Aotus subjects; such high gametocyte levels occurred in half of the animals that infected mosquitoes. Dissection data (from individual Aotus that yielded positive lots) varied for each series of monkeys, but lot totals again indicated a greater consistency of positive feedings from the sporozoite induced infections and splenectomized hosts. The number of infected mosquitoes in positive lots were both highest and lowest from monkeys with trophozoite induced infections, viz., 32% of 832 dissected for splenectomized Aotus donors and 17% of 243 for the intact Aotus. Respective mean oocyst counts, 9.1 and 2.7, also were higher and lower than for the sporozoite induced infections. According to the foregoing results, it was apparent that variability in host influence (between individual monkeys) was sometimes a greater factor in determining gametocyte infectivity than the type of induced infection.

The Achiote strain proved to be vector infective from all experimental host species except Saguinus during the inclusive trials; however the marmoset was shown under previous contracts to support infective gametocytemias. Findings with these other models did not indicate a higher gametocyte infectivity from donors infected by sporozoites, although fewer animals were used or generally poor vector infections were realized from both types of induced parasitemias. Many of the mosquito infections were obtained at low male gametocyte indices (<100 per cmm).

During the above, A. albimanus were fed on hosts showing several parasitemia episodes. The results of these trials (Table 15) did not reveal significant or consistent differences in infectivity between the primary attack and relapse at comparable gametocyte levels in the same monkey. The infections in monkeys

which yielded high percentage of positive mosquitoes during the first attack, also did during relapse. The ratio of positive to total numbers of dissected mosquitoes is small, as negative feedings are included in the data.

In experiments with newly colonized Escobal A. albimanus, no significant differences appeared in susceptibility comparisions with the GML strain (Table 16). Paired lots were fed 219 times on 43 Aotus infected with Achiote P. vivax. In midgut and salivary gland dissections, from 15 hosts yielding positive mosquitoes, 5.35% of 1,719 specimens of the GML strain were infected versus 5.56% of 1,710 Escobal specimens. Respective mean oocyst counts were 8.37 (65 positive midguts) and 9.75 (51 positive midguts). A total of 24 companion feedings on 5 Saimiri and 3 A. fusciceps did not produce infections in either strain of A. albimanus. The findings indicate that although our standard line of A. albimanus has been maintained in colony for more than 35 years, its vector competence has been retained comparable to wild native population.

For Achiote vivax, companion feedings of A. albimanus and A. aztecus resulted in the former being more infected from A. trivirgatus and A. fusciceps while the latter yielded more infected lots from S. sciureus and S. geoffroyi (Table 17); totals of feedings on all animals indicated a similar susceptibility (16% vs 15%). These data were based on tabulations from infected lots only. Notwithstanding the above, A. albimanus is the preferred experimental vector, due to the relatively short mean life span of A. aztecus adults in the laboratory. In the latter case, the number of females surviving through the sporogonic cycle is more restricted. Among other anophelines tested against A. albimanus for Achiote vivax susceptibility, 4 (A. neivai, C. bathana, A. eiseni, A. triannulatus) were used for the first time in these systems (primarily Aotus). In most cases, the test anophelines were not available on a continual basis, consequently few infected mosquitoes were obtained for valid comparision. Only lot infections of A. triannulatus were significant, as high as 67%, approximating the levels seen in A. albimanus.

Heavy parasitemias of the highly virulent Vietnam-Palo Alto vivax were produced in Aotus, but mosquito feedings (69 lots) of A. albimanus and in some cases A. aztecus on all hosts did not show oocysts or sporozites at dissection. Significant gametocyte counts also were seen in Saguinus, which is unusual in consideration of our findings with the Achiote strain in this donor; however, there were no infections for A. albimanus in 19 trials.

A total of 49 lots of A. albimanus and A. aztecus were fed upon Aotus, Saguinus and Saimiri infected with the Rio Meta, Colombia strain of P. vivax. No oocysts were detected, although circulating male gametocytes (in one Saguinus) exceeded 600 per cmm.

A combination of 4 indigenous strains of P. brasilianum in A. fusciceps and A. geoffroyi were examined for their vector infectivity as indicated in Table 18 (Cerro Nique, Cerro Quia, Cerro Azul, Rio Chico). Routine diurnal feedings gave positive mosquitoes (oocysts) only with the Cerro Nique strain. Parasite levels were increased by splenectomizing 2 A. geoffroyi hosts bearing the naturally acquired Cerro Azul and Rio Chico parasites. During high gametocytemias that followed in these monkeys, A. albimanus and/or A. aztecus (a proven experimental vector of P. brasilianum) were exposed at 2 to 4 hr intervals throughout the diurnal period and night, thus encompassing the hours we have found naturally-associated anophelines to take a blood meal. Only one low grade infection then was obtained, in A. aztecus. These results are similar to ours previously evidenced with vivax and falciparum malarias in Aotus and Cebus monkeys, in that we have been unable to confirm, in the New World models, reports on diel changes in gametocyte infectivity of Old World plasmodia.

The Aotus adapted strain of P. brasilianum (PB-A), while producing substantial gametocytemias in blood induced passage lines (especially Aotus), proved to be non-infective for 67 pools of A. albimanus or A. aztecus.

A line of the Cerro Quia strain was made resistant to pyrimethamine in an attempt to increase gametocyte infectivity. Both the resistant line and its sensitive counterpart were compared using the same 2 anophelines as above. Some 150 lots (35,000 mosquitoes) were tested in periods before, during and after the induced pyrimethamine resistance, but all midguts and salivary glands screened were negative.

A single P. simium isolate adapted to Panamanian monkeys was tested for its efficacy in transmission systems, with a total of 327 lots and 5 species of anophelines (Table 19). Three Aotus yielded infected A. albimanus mosquitoes to the oocyst stage, while only one of these monkeys showed a significant male gametocytemia (2,862 per cmm). Repeated feedings on the same hosts did not obtain additional vector stages. As indicated, 4 other mosquito species were tried on Aotus without positive results. The remaining monkeys tested also were not effective for transmission studies.

Concomitant with dissections for malarial parasites, the anopheline mosquitoes routinely were examined (head, thoracic musculature and midgut) for other blood parasites of monkeys (filaria and trypanosomes). Additionally, Culex aikenii (1,400 females) and C. pipiens quinquefasciatus (2,000 females) and in limited numbers a phlebotomine sandfly, Lutzomyia sanguinaria, were tested, primarily on Saguinus hosts that showed high microfilaremias. Such attempts to demonstrate the vector stages failed, and we have been unable to duplicate our finding under a previous Army contract of an infective stage larva (Dipetalonema ?) in the thoracic musculature of A. albimanus acquired during an Aotus feeding.

D. Transmission

All sporozoite induced infections were effected with A. albimanus and the Achiote strain of P. vivax. Limited trials with A. triannulatus, and the same parasite strain, did not yield infected Aotus recipients.

Transmission experiments were attempted using 147 A. albimanus lots having acquired infections from 80 Aotus and 5 A. fusciceps; one or more positive recipients resulted with the mosquitoes in 62 (42%) of these lots, derived from 39 (49%) of the animals. The donor monkeys constituted the 33rd through 135th serial transfer of the Achiote strain, and sporozoite infectivity did not diminish upon repeated trophozoite passage nor improve consistently after each vector cycle. Transmission success from a given animal was demonstrated by as many as 4 of 5 lots obtained on consecutive days, although up to 6 such trials with other individual monkeys failed. While the mosquito stages originated from hosts during primary attack or relapse at varying parasitemias, their infectivity was confirmed only in the former phase. Sporozoite viability was comparable using both intact and splenectomized donors. Irrespective of the route of sporozoite introduction (interrupted bite, intravascular, intrahepatic or intraperitoneal inoculation), for some series parasitemias appeared in the majority of recipients and others (involving up to 13 animals, and representing 2 or more species) produced none. Sporozoite plus ratings in successful experiments ranged from 9 to 482 ($\bar{x}$=100), and as few as 4 mosquitoes ($\bar{x}$=36) infected monkeys. Positive results were not predictable on the basis of high plus ratings or the numbers of infected mosquitoes. Sporozoites harvested over a range of 11 to 20 days of extrinsic incubation, at 24°C, were infectious. On several occasions, without deleterious effects, the mosquitoes were transferred to a lower temperature (22°C) the final 24-72 hours.

Sporozoite transmissions were achieved for each of the 5 species of New World monkeys proven susceptible to the trophozoite stages. The tests, with 529 inoculated monkeys, are presented by host alteration in Table 20. All A. geoffroyi became infected, while the poorest frecuency was demonstrated by Aotus. Ratios of positive to total animals were appreciably higher in splenectomized than intact subjects only among Saimiri and Saguinus, but not at significant levels. Meaningful differences also could not be established for Imuran(R)-treated (versus untreated) monkeys of both categories, which overall developed infections as follows: 3 of 7 A. fusciceps, 2 of 11 S. geoffroyi, and 7 of 47 A. trivirgatus. Intraspecific differences were especially apparent for Saimiri; 4 were susceptible when inoculated a second time and others did not show a parasitemia until incorporation into a 6th experiment. Certain Aotus also were inoculated up to 6 times (with separate mosquito pools) without becoming infected, although sporozoite viability was confirmed by parasitemias in companion animals.

Aotus were employed as co-recipients for the majority of tests, and served as a standard to additionally evaluate the utility of each model. As shown in Table 21, each of these series of experiments yielded proportionately more members of the other species infected than Aotus. The observed differences, from Aotus, for A. geoffroyi and A. fusciceps were statistically significant ($P = < 0.5$ and < 0.02, respectively, by Chi square analysis).

Trophozoite passages were initiated from sporozoite infected animals, leading to a maximum of 13 random transmissions in a single line. Consecutive cycles between normal monkeys were realized from Aotus to another Aotus, to an A. fusciceps, and to 5 Saimiri. At the 110th blood or sporozoite passage, a human subject (JA) developed parasitemia 10 days after inadvertently receiving bites of A. albimanus (ex Aotus), proving the retention of infectivity for man after adaptation in monkey systems for more than 5 1/2 years. Positive transmission from JA then followed to an A. geoffroyi and an Aotus (exoerythrocytic forms only).

DL-methionine, a lipotropic agent, was administered in trials to improve the probability of establishing sporozoite induced infections (Table 22). The Guatemalan A. geoffroyi subjects were inoculated from a common sporozoite pool, 7847 treated and 7846 serving as its control. Two specimens representing the indigenous subspecies, 5639 and 7181, also were respective test and control intact animals that received another sporozoite pool. As shown, initiation of patency in the A. geoffroyi monkeys given methionine was markedly shorter than for their controls, which had unusually

protracted incubation latencies of 196 and 133 days. The presumptive action of the compound could not be verified in replicates with other species; a treated Saimiri experienced a longer prepatent interval than its untreated control (58 vs 12 days), while only the control became positive for 2 experimental A. fusciceps and infections did not appear in 3 treated and 1 untreated Aotus.

In other transmission series, with untreated recipients, 4 splenectomized A. geoffroyi evidenced parasitemias by the 25th day and experienced higher parasite densities (> 1,000 per cmm) than intract A. geoffroyi subjects. Multiple attacks and varying subpatent periods occurred in the 2 groups. Primary patency was longer than relapses for a given monkey, with the exception of a 96-day parasitemia that ended 358 days after inoculation. Upon relating the infections in A. geoffroyi to those for A. fusciceps, an overlap of values was apparent, although splenectomy effected considerably higher parasitemias (to 28,000 per cmm) in the latter species.

Among the Saguinus marmosets, again there was a great diversity of primary attack and relapse characteristics for normal and altered animals. The shortest and longest prepatent periods (15-70 days) were seen in recipients that had been both splenectomized and treated with Imuran(R). Low grade development (< 10-110 per cmm) as well as the heaviest infections (to 35,170 per cmm on the 141st day, during ascent) also were recorded from splenectomized hosts. Although 4 of 5 marmosets succumbed in the course of patency, observation periods extended to 294 days.

Prepatent intervals averaged more than a month for Saimiri and were virtually within the same ranges as Saguinus infections. The length of patency was comparable to other host systems, and in one splenectomized Saimiri persisted 137 days. Infection differences between intact and splenectomized Saimiri were most apparent in the mean peak counts of the initial attack (4,561 vs 24,634 per cmm). Only low grade development resulted at relapses. However, each of the 4 re-infected Saimiri, exposed a second time to sporozoites up to 1 and 1/2 years after the disappearance of parsites from the peripheral blood, showed ensuing parasitemias that approximated or surpassed densities reached after the initial inoculation; the heaviest episodes were in 3 splenectomized prior to the second challenge.

The shortest onset of patency (from 8 days) and the severest parasitemias (self-limiting at 68,160 per cmm) for transmitted infections have been from intact Aotus. Marginal infections were evidenced with some Aotus monkeys, including those that had been

splenectomized. Relapses occurred in all of 5 examined beyond the initial attack, yielding markedly lower densities among the normal animals. Subpatent periods (to 90 days) in intact Aotus were longer than those experienced by intact subjects of the other species, although splenectomized Ateles and Saimiri showed intervals of more than 100 days. A sixth parasitemia for one Aotus terminated 483 days after inoculation, exceeding persistence in other models by at least 3 months.

Our observations show that susceptibility of an individual was a greater consideration for establishing infections than the route or number of sporozoites inoculated. Moreover, the degree of sporozoite viability appeared to be of equal or more importance in provoking a patent parasitemia, except for A. geoffroyi. We suggest that this could be a reflection of the source of parasites (the donor and its infection) plus the vector related factors. Our earlier reports, without reference to concomitant sporozoite viability, have indicated that good mosquito infections of P. vivax are obtained from certain monkeys even at low gametocytemias and more regularly from Aotus if harboring transmitted infections.

Plasmodium vivax can be maintained readily in the 5 species of monkeys by blood inoculation, yet transmissions could not be achieved reliably or uniformly with known infective sporozoite preparations. The effect of the immunosuppressant was equivocal, and splenectomy was not consistently advantageous. Our studies with DL-methionine did show an apparent activity in the vivax-A. geoffroyi system.

E. Delineation of Exoerythrocytic Stages

As part of the total evaluation of monkeys as models for human malaria, extensive efforts have been directed torward the study of exoerythrocytic stages (principally P. vivax).

Seven species of indigenous nonhuman primates, the Peruvian Cebus apella, and Guatemalan A. geoffroyi were utilized (Table 23). A total of 64 biopsies represented 35 subjects.

In tests with Panamanian A. geoffroyi, exoerythrocytic (EE) stages were seen in 7 and 9-day biopsies from co-recipients. The EE forms from the animal administered methionine were larger in size than these found in the corresponding control biopsy (Table 23), and at 7 days the size difference was statistically significant. The early bodies were round or elliptical and contained relatively large and irregularly shaped nuclei. Cytoplasmic clefts were apparent in several instances. The 9-day stages were lobed, with

finely divided nuclei, and showed merozoite development. A 209-day biopsy was performed on the untreated animal, 5 days after a relapse was initiated, and yielded a suggestive degenerate form which requires confirmation.

Among the A. fusciceps, 3 of 4 produced EE stages and the same hosts developed patent infections. The 7-day bodies were circular or ovoid in appearance, ranging in maximum diameter from 15.0 μ to 25.0 μ. The number of nuclei at this stage of development was variable, 4 to 30 per section. The nuclei were deeply stained, and in most cases were of uniform size, discrete and rounded. The cytoplasm was slightly granular, compact, with several small, clear vacuoles in approximately half of the parasites. Shrinkage from the host cell was noted in a few instances. Compression of the host cell nucleus occurred, and there was no apparent tissue reaction in the surrounding area. The 10-day exoerythrocytic stages were lobed, with largest diameters measuring 63.8μ and 67.5μ. Nuclei were finely divided, irregularly shaped and not clearly defined. The cytoplasm was extremely granular and diffuse; beginning cleft formation was apparent. Small vacuoles also were present in one body. There was moderate shrinkage of the parasite at 10 days from the host cell and again no evidence of reaction in the surrounding tissue.

In experiments with 6 Saimiri hosts, EE development was demonstrated in 4; subsequent patent infection developed in 2 of these. At 7 days, in one animal a single, relatively small, parasite was evidenced while normal growth occurred in the other. The bodies were circular or ovoid, containing deeply stained, large, irregularly-shaped nuclei. The cytoplasm was loose to compact, usually with several small vacuoles, and cleft formation was seen. Considerable variation in morphology was observed at 9 days. Although most of the parasites were ovoid, others were irregular with incipient lobing. As in other biopsies, shrinkage was common, being moderate to severe. The 9-day body growth varied from that seen at 7-days to complete merozoite formation in the initial state of rupture. Most were well stained with compact, granular cytoplasm. At 10 days, the bodies were rupturing, with release of merozoites into the surrounding tissue. Among these 10-day parasites still structurally intact, some possessed deeply stained aggregations of merozoites, with half the bodies showing 1 to 5 rather large vacuoles (10.0μ). At this biopsy, there were unusual tissue forms, distinguished by 2 or 3 large (6.3 to 23.8μ) round to avoid masses, which occupied most of the cytoplasm. Such areas were opaque, dark violet in color, and contained numerous particles somewhat larger than the normally developing merozoites. Under light microscope magnification of 1,000X, a membrane appeared to surround each mass, with shrinkage from the cytoplasm.

This atypical morphology warrants further study.

Although no patent parasitemia occurred after intrahepatic sporozoite inoculation into C. capucinus and C. apella (5 monkeys), tissue stages have been demonstrated in 4 of them, at 7, 9 and 10 days. The majority of the EE parasites were typical of those seen in recipients representing other host species that did develop patent infections. In one C. capucinus (4894), abundant 9-day forms, uniformly developing, were larger and more mature than those from hosts susceptible to P. vivax. There was a notable absence of vacuoles, and lobing was prominent. Growth of the bodies was slower at 9 days in the other Cebus subjects, but t-test comparisons (between 4894 and 5293) of length, width, and length/width ratio did not indicate statistical significance of the differences. The stages in C. apella (a South American species) were much smaller at comparable days and appeared to be degenerating at day 9. Blood subinoculation between 9 and 14 days after transmission from 2 Cebus, positive for the EE stage, did not produce infections in Aotus recipients. All attempts failed to establish EE schizonts in Alouatta, the only other New World monkey besides Cebus in which we have not been able to induce blood infections of P. vivax; blind passages to Aotus from 3 of the Alouatta in these experiments also failed to initiate an infection.

Upon examination of the Saguinus material, a single schizont of the Achiote strain was identified from a 7-day biopsy; a 9-day sample from the same host, the only subject inoculated in that trial, was negative and a patent parasitemia did not occur over a 19-day period. The EE body, measuring 14.0 X 17.5μ and evidencing shrinkage, was lightly stained in relation to surrounding tissue. The cytoplasm was vacuolated, evenly distributed, and contained several small, dark granules. Although its size was within the range we have seen at 7 days in New World cebid monkeys, the morphology suggested arrested growth. Among the remaining marmosets, none yielding EE or blood stages, one was euthanized at 7-days, and 5 were followed for 33 to >100 days. Infectivity of sporozoite inocula in 2 of these tests was verified by patency in the companion Saimiri and Ateles, and in Saimiri (at 9 days) by the presence of the hepatic phase.

In limited trials with Aotus, an 11-day tissue form was found. The body was oblong, with rounded, small nuclei and although not appearing to be degenerating, a patent infection did not follow in this host.

Previous to the above findings the pre-erythrocytic phase of P. vivax in monkeys had been observed by other investigators

only in Aotus (at 6, 7 and 8 days). Our results show that other New World primate species may offer greater utility for such studies.

Plasmodium falciparum sporozoites, derived directly from a human source, were inoculated intrahepatically into 2 C. capucinus; biopsies at 8 and 12 days, respectively, were negative and patent infections were not initiated.

III. Cooperative Activities

Numerous projects in conjunction with investigators from other laboratories were initiated during the period of this report or continued from the previous contract. The availability of various biological material from Panama afforded an unique oportunity for study, utilizing techniques not immediately available at GML and for gaining needed information apart from our mission in this program. A brief description of the objectives and/or results of the cooperative efforts follows:

Dr. H. Fremount, East Stroudsburg State College, Pennsylvania completed work on the sequestration of P. falciparum (Uganda-Palo Alto strain) in Saimiri. The distribution and ultrastructure of parasitized red blood cells in various organs was evaluated at peripheral parasitemias of less than 2 percent. Schizonts were trapped principally in the splenic sinuses and to a lesser degree in the microcirculation of the bone marrow and cardiac venticle. An ultrastructural abnormality was present in the membrane of infected red cells which appeared as an electron dense, knob-like protrusion. Similar work is being completed using the P. vivax- Saimiri model. Liver biopsy material, prepared for electron microscopy, subsequently was sent to Dr. Fremount; 9 samples (at 5-10 days) were obtained from 2 Saimiri, one Cebus and 2 Ateles. The results are pending.

The helminth and trypanosome data (as summarized in a previous section) were acquired during continuing intra-laboratory collaboration with Dr. O. Sousa, Parasitology Department.

The collection and associated studies on jungle anophelines were conducted, in some cases, with Dr. M. Boreham, medical entomologist of the Canal Zone Government. Additionally, Dr. Boreham was furnished Ateles monkeys as sentinel animals in developing mosquito trapping techniques.

Adult A. albimanus mosquitoes were supplied to the Environmental Health Laboratory, Canal Zone for residual insecticide

testing and colony establishment, and to the National Malaria Eradication Unit, Panama for insecticide application trials. A. albimanus, A. aztecus, and C. p. quinquefasciatus larvae were provided for field and indoor experiments in testing susceptibility to an insect growth regulator, conducted by Mr. Larry Senior, Zoecon Corporation, Palo Alto, California and Dr. Charles Schaefer, Mosquito Control Research Laboratory, Fresno, California. Anopheline and culicine adult preserved specimens also were furnished to R. Lee, University of Alberta, Canada, who is engaged in fine-structure studies on the mouthparts of mosquitoes.

Electrophoretic patterns of blood samples of A. geoffroyi, A. fusciceps and a hybrid of these species have been evaluated by Dr. M. Goodman, Wayne State University, Detroit, Michigan. While there were no marked differences among the patterns, it was indicated that the carbonic anhydrase pattern may offer a potential for the differentiation.

Biological material was supplied to two investigators at the New England Regional Primate Center, Southborough, Mass. Dr. T. C. Jones cultured skin biopsies of Panamanian Ateles in attempts to distinguish the species by chromosome patterns. Dr. H. Baharonna obtained blood and kidney cell cultures, also from Ateles, for viral isolation; herpes virus was found, and the characteristics and incidence of the virus determined. Other Ateles skin biopsies were sent to Dr. Bernischke, San Diego Zoo, for culture and chromosome studies. Alouatta blood samples were prepared for Dr. S. Boyer, Johns Hopkins Medical School, Baltimore who is evaluating enzyme components.

The Rio Meta, Colombia strain of P. vivax was given to Dr. Collins, Unit of Primate Malaria, Chamblee, Ga. primarily for vector infectivity surveys. Cryopreserved P. falciparum parasites were sent for antigen preparation to Dr. A. Corredor Arjona, Ministry of Public Health, Bogota, Colombia. P. brasilianum infected blood was forwarded to Dr. Huldt, Karolinska Institute, Stockholm, Sweden for chronic infection work with Saimiri.

Several shipments of monkeys were effected, pursuant to cooperative arrangements: A. fusciceps to Dr. Collins, Central American Research Station, San Salvador, El Salvador, for onchocerciasis research and also to Dr. H. Rassi, Caracas, Venezuela, and Aotus monkeys to Dr. A. Voller, Nuffield Institute of Medical Research, London, England in attempts to re-establish a strain of monkey adapted P. malariae. We have additionally provided Dr. Voller with frozen strains of P. vivax, P. falciparum, and P. brasilianum, as well as non-infected erythrocytes from Aotus and Ateles.

Dr. S. Kalter, Southwest Foundation for Research and Education, San Antonio, Texas has surveyed sera from malaria free monkeys (Aotus, Saguinus, Ateles, Saimiri and Cebus) with the following results: no titers were found for SA 8, parotitis, influenza, parainfluenza, SV5, or SV41 virus. An Ateles showed a titer of 1:40 for Herpes hominis and a Cebus had a titer of 1:160 for H. tamarinus. In other tests against Reo 2 and 3 and rubella, Aotus demonstrated high titers (1:320). Titers of 1:160 and 1:320 against monkey pox and vaccinia were recorded in A. fusciceps, A. geoffroyi, Cebus and Alouatta.

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Table 1

Development of Plasmodium falciparum (Panama II strain) in normal Panamanian Aotus trivirgatus.

Monkey no.	Passage no.	Prepat. (subpat.) pd. - days	Pat. pd. - days		Max parasitemia per cmm	
			primary	recrudescence	primary	recrudescence
6901	1	1(46)	6	41	120	55,130
7075	3	1	16	-	490	-
7084	4	1(22)	13	21	3,020	1,270
7088	4	36(18)(19)	3	58;21	<10	2,690;112,650
7177	4	1(44)	5	20*	70	57,760
7092	5	4(21)	8	28*	50	29,890
7195	5	1(12)	13	10*	1,080	1,170
7237	5	4	31*	-	7,710	-
7089	6	1	14*	-	5,790	-
7245	10	1	12*	-	694,310	-
7267	11	1(24)	15	19*	23,910	1,008,000
7297	12	6	21*	-	160	-
7323	12	1(29)	3	16*	10	133,330
7428	14	1	10*	-	1,542,320	-
7444	15	1(18)	15	9*	91,130	15,260
7466	16	1	44*	-	1,156,600	-
7445	17	6	18*	-	120,750	-
7496	18	1	24*	-	84,410	-
7612	18	8	17*	-	410,700	-
7475	19	3	65*	-	511,940	-
7934	19	7	44*	-	416,500	-
7567	20	1	5*	-	89,440	-
8069	20	8	19*	-	101,120	-

* Died during patency.

Table 2

Infections of *Plasmodium falciparum* (P II strain) in *Alouatta villosa trabeata*.

Monkey no.	No. parasites inoc. x 10^6	Prepatent or (subpatent)pd. days	Patent pd. days	Max. parasit. per cmm	Max. parasit. pat. day	Total period examined days
8008	738	1	5	<10	-	
		(54)	16	10,680	11	
		(21)	45	6,420	42	
		(13)	20	7,220	12	238
8009	0.8	5	18	14,910	16	
		(31)	15	1,060	13	
		(37)	25	5,490	11	166
8011	21	11	16	35,060	8	
		(19)	21	14,180	7	
		(6)	12*	33,060	12	85
8013	70	17	20	5,820	7	
		(11)	47	4,600	40	
		(30)	3	120	1	
		(25)	21	3,370	7	203
8014	4	19	22*	6,520	6	41

* At death following splenectomy.

Table 3

Development of Plasmodium falciparum (Vietnam-Oak Knoll Strain) in Aotus trivirgatus and Saimiri sciureus.

Monkey no.	Passage level no.	Prepatent pd.-days	Patent pd.-days	Max. parasit. per mm^3	Day to reach 100,000/cmm	Remarks
Aotus trivirgatus						
6135	1	3	22‡	437,440	6	
6160	2	6	42‡	102,120	10	
6150	3	6	11‡	642,600	6	
5288	4	6	22	635,120	6	3 recrudescences
6182	5	19	26	413,280	5	
6244	6	8	26‡	405,660	6	
6207	7	5	23	206,490	7	
6248	8	4	33	278,000	8	
6261	9	3	37	466,020	9	
6213	10	3	31	1,187,000	5	
6243	11	6	16‡	483,920	4	
6437	12	4	11‡	561,190	6	
6468	13	2	11‡	1,559,880	6	
6496	14	9	10‡	1,005,560	5	
6607	15	4	12‡	1,140,430	6	
6597	16	4	21‡	401,030	6	
6661	17	6	12‡	2,018,500	8	
6658	18	3	12‡	665,280	7	
6698	19	4	10‡	1,191,950	6	
Saimiri sciureus						
4684	7	6	10	210	7*	Neg. 184 days post patent pd.
5203	7	6	17	5,610	8*	Neg. 193 days post patent pd.
6620	17	4	15	13,140	5*	Neg. 53 days post patent pd.

‡ Died during patency.

* Patent day of maximum parasitemia.

Table 4

Plasmodium simium infections induced in six species of Panamanian monkeys.

Monkey no.	Parasites inoc. x 10^6	Prepatent (subpatent)pd. days	Patent pd. days	Max. parasitemia per cmm	Max. parasitemia patent day
Saguinus geoffroyi					
6029	16	1	22*	200,360	14
6486	3	9	15*	264,650	10
Alouatta villosa					
7204	21	12	23	42,530	11
Cebus capucinus					
5294	217	23	26	2,950	15
4890	4	42	23	1,590	14
4896+	217	-	-	-	-
Ateles fusciceps					
5828	5	19	21	230	14
5828R	-	(32)	7	20	6
Ateles geoffroyi					
7097	5	13	23	220	14
7097R	-	(48)	16	30	11
Saimiri sciureus					
7137	36	3	5	60	3
6960	2	1	21	30	16
6960R	-	(6)	21	17,660	13
6832	23	32	7*	<10	-

* At death.

\+ Splenectomized prior to inoculation.

R Recrudescence.

Table 5

Plasmodium simium infections in *Aotus trivirgatus*.

Monkey no.	Passage level no.	Prepatent pd. days	Patent pd. days	Max. parasitemia	
				per cmm	patent day
5698	1	4	87*	77,720	17
6653	2	8	33*	71,880	15
6746	2	3	10*	259,740	10
6685	3	1	27*	217,590	13
6767	5	3	9*	143,090	9
6923	5	14	29*	118,760	10
6896	6	1	3*	180	3
7015	6	2	42*	28,230	23
7045	7	4	3*	<10	-
7065	8	1	15*	298,290	15
7094	9	5	34*	355,960	13
7197+	9	8	9*	1,140	7
7096	10	1	26	99,730	12
7232	11	2	51*	17,940	11
7227	12	2	17*	113,760	11
7348	13	5	40*	40,130	14
7599+	13	9	10*	12,140	10
7381	14	3	17*	302,400	11
7406	14	5	13*	290,620	10
7521	15	1	13*	847,190	13

* At death.

\+ Inoculated with frozen blood.

There was no relationship between the number of parasites inoculated and the prepatent period.

Table 6

Infectivity of an Aotus-adapted strain of Plasmodium brasilianum (PB-A) for Panamanian monkeys.

Monkey no.	No. parasites inoc.X10^6	Prepat pd.days	Patent pd. days	Maximum parasitemia per mm^3	Maximum parasitemia pat.day	Remarks
			Aotus trivirgatus			
7925	32(frozen)	7	129*	7,990	29	
7927	32(frozen)	15	43*	102,120	21	
			Cebus capucinus			
5290	4.4	25	88	317,460	36	Splenectomized patent day 13. Chloroquine (10mg/kg) for 5 days, patent day 90.
			Saguinus geoffroyi			
7977	24	1	52*	3,060	25	
			Saimiri sciureus			
7370	1.2	3	4	<10	-	Negative 64 days.
8000	1.6	-	-	-	-	Died day 18 post-inoculation.

* Died during patency.

Table 7

Frequency of trypanosomes and microfilariae in the squirrel monkey, Saimiri sciureus

District	Total examined	% positive	Micro-filariae (%)	Trypano-somes (%)
Chiriqui Province				
Alanje	414	11.7	9.7	1.9
Bugaba	43	16.3	16.3	0.0
David	13	0.0	0.0	0.0
Baru	2	0.0	0.0	0.0
Total	472	11.6	9.9	1.7

Table 8

Frequency of trypanosomes (T) and microfilariae (M) in the marmoset, Saguinus geoffroyi

District	Total examined	% positive	M (%)	T (%)	Trypanosoma cruzi (%)	Trypanosoma rangeli (%)	Trypanosoma minasense (%)
Panama Province							
Arraijan	60	98.3	81.7	90.0	9.3	79.6	74.1
Capira	19	89.5	73.7	78.9	20.0	86.7	46.6
Chame	1	100.0	0.0	100.0	0.0	0.0	100.0
Chepo	27	92.6	81.5	48.1	23.1	61.5	61.5
Chorrera	142	88.0	66.9	80.3	18.4	85.1	50.8
Panama	152	85.5	73.7	50.0	23.7	82.9	40.7
Canal Zone	1	100.0	100.0	0.0	0.0	0.0	0.0
Colon Province							
Colon	6	83.3	83.3	83.3	0.0	80.0	80.0
Total	408	88.9	73.0	68.1	12.2	55.8	36.5

Table 9

Colony births of Cebus capucinus.

Female	Deliveries
Cebus capucinus imitator	
	Group 1
4902	24 April 1970(M)*; 3 July 1972 (M); 8 February 1974(M)
4885	20 May 1971(F)
4889	9 June 1971(F); 5 September 1972(M)
4901	8 February 1972(M)
NR	17 March 1975(unsexed)
	Group 2
5298	1 June 1971(M); 15 October 1972(unsexed)
NR	13 December 1973(unsexed)
Cebus capucinus capucinus ‡	
684	December 1968(F); 24 April 1970(F); 1 July 1971(F); 6 May 1972(unsexed)+
206D	12 March 1971(M)
NR	9 May 1969(F)
NR	9 April 1971(F)
NR	11 April 1971(M)
NR	14 January 1974(F)
NR	27 February 1974(M)

M Male.
F Female.
* Conceived prior to captivity.
NR Not recorded.
‡ Data not recorded for two additional infants, mother(s) unknown.
+ Mother and fetus died during parturition.

Table 10

Malaria dissection records and parous rates

for Anopheles neivai and Chagasia bathana

from evening arboreal biting collections

in Panama forest study areas.

(1971 and 1972)

	Panama Province	Darien Province	Combined totals
Inclusive periods	Late wet season (Dec. - Jan.)	Dry season (Mar. - Apr.)	
No. canopy stations	4	10	14
Man - bait collections	30	83	113
Man hours	42	105	147
Anopheline captures (♀♀)			
A. neivai	55	321	376
C. bathana	0	69	69
Malaria dissections			
A. neivai total	17	305	322
Midguts exam.	17	149	166
Saliv. gl. exam.	17	283	300
C. bathana total	-	67	67
Midguts exam.	-	64	64
Saliv. gl. exam.	-	65	65
Age grading			
A. neivai total	11	169	180
Nullipars	6	90	96
Pars (%)	5(45)	79(47)	84(47)
C. bathana total	-	50	50
Nullipars	-	25	25
Pars (%)	-	25(50)	25(50)

Table 11

Incidence of blood parasites in monkeys from Darien camp region during concurrent vector investigations.

Monkey Species	No. Animals Examined	Positives		
		Plasmodium brasilianum	Microfilariae	Trypanosomes
Ateles fusciceps	72	11	46	9
Alouatta villosa	27	4	0	13
Cebus capucinus	10	1	6	1
Aotus trivirgatus	4	0	0	0

Table 12

Experimental vector species used in malaria and associated investigations at Gorgas Memorial Laboratory (1971-75).

Species	Laboratory Utility
Anopheles albimanus	
GML-Rozeboom strain -	Colonized/ Served as standard vector
Escobal strain -	Colonized/ Vector competence trials
A. aztecus	Colonized/ Vector competence trials
A. oswaldoi	Colonized 5 generations/ Vector competence trials
A. triannulatus	Rearing studies/ Life span-ovarian development/ Vector competence trials
A. aquasalis	Rearing studies/ Life span-ovarian development
A. punctimacula	Rearing studies/ Vector competence trials
A. eiseni	Rearing studies/ Vector competence trials
A. pseudopunctipennis	Rearing studies/ Vector competence trials
A. squamifemur	Life cycle data/ Description of all immature stages
A. neivai	Life cycle data/ Description of egg stage/ Vector competence trials
Chagasia bathana	Life cycle data/ Reared consecutive generations via force mating/ Description of egg stage/ Vector competence trials
Culex p. quinquefasciatus	Colonized/ Trypanosome - helminth vector competence trials
C. aikenii	Trypanosome - helminth vector competence trials
Lutzomyia sanguinaria	Trypanosome - helminth vector competence trials

Table 13

Anopheline mosquito tests for susceptibility to strains of *Plasmodium falciparum* and *P. malariae* according to donor system.

Malaria strain	Host	Anophelines tested negative	Anophelines infected
		P. falciparum	
Panama II	*Aotus trivirgatus*	*A. albimanus* *A. aztecus* *A. triannulatus* *A. oswaldoi* *A. punctimacula*	
	Saimiri sciureus	*A. albimanus* *A. aztecus*	
Vietnam-Oak Knoll	*Aotus trivirgatus*	*A. albimanus*	
Malayan Camp	*Aotus trivirgatus*	*A. albimanus*	
Guayabalito-4	Man*		*A. albimanus*
Turbo (Colombia)	Man*		*A. albimanus*
		P. malariae	
Don Bosco	Man*	*A. albimanus*	

* Naturally acquired infections.

Table 14

Anopheline mosquito tests for susceptibility to strains of *Plasmodium vivax* according to donor system.

Malaria strain	Host	Anophelines tested negative	Anophelines infected
Achiote	*Aotus trivirgatus*	*A. oswaldoi* *A. punctimacula* *A. neivai* *C. bathana* *A. eiseni* *A. pseudopunctipennis*	*A. albimanus* *A. aztecus* *A. triannulatus*
	Saimiri sciureus	*A. oswaldoi*	*A. albimanus* *A. aztecus*
	Ateles fusciceps	*A. aztecus* *A. oswaldoi* *A. punctimacula*	*A. albimanus*
	Ateles geoffroyi		*A. albimanus*
	Saguinus geoffroyi	*A. albimanus* *A. aztecus*	
	Man	*A. aztecus*	*A. albimanus*
Rio Meta (Colombia)	*Aotus trivirgatus*	*A. albimanus*	
	Saimiri sciureus	*A. albimanus* *A. aztecus*	
	Saguinus geoffroyi	*A. albimanus*	
Vietnam-Palo Alto	*Aotus trivirgatus*	*A. albimanus* *A. aztecus*	
	Saguinus geoffroyi	*A. albimanus* *A. aztecus*	

Table 15

Infectivity of *Plasmodium vivax* to *Anopheles albimanus*; a comparison of primary attack versus relapse feedings*

Parasitemia episode	Male gamet. range-per cmm	Lots Pos./Tot.(%)	Mosquitoes Pos./Tot.(%)	Midgut dissections Pos./Tot.(%)	Oocysts Range	Oocysts $\bar{x}$
Aotus trivirgatus (5 subjects)						
Primary attack	<10-400	13/40(32.5)	92/735(12.5)	66/714(9.2)	1-41	6.5
Relapses	<10-401	16/58(27.6)	73/1,131(6.5)	44/1,115(3.9)	1-25	4.4
Ateles fusciceps (3 subjects)						
Primary attack	<10-760	9/86(10.5)	27/1,765(1.5)	26/1,756(1.5)	1-7	2.2
Relapses	<10-471	5/44(11.4)	17/914(1.9)	16/914(1.8)	1-18	3.2
Ateles geoffroyi (1 subject)						
Primary attack	<10-49	2/20(10)	2/400(0.5)	2/400(0.5)	1	1.0
Relapses	<10-72	0/18(0)	0/352(0)	0/352(0)	-	-

* Data summarized from hosts yielding infected mosquitoes; other similar trials, involving 8 *Aotus*, 1 *At. geoffroyi*, 5 *Saguinus geoffroyi*, and 4 *Saimiri sciureus*, did not produce infected *A. albimanus* from multiple parasitemias.

Table 16

Comparison of susceptibility for the GML (Rozeboom) and Escobal strains of *Anopheles albimanus* to Achiote *Plasmodium vivax*.

Results from paired feeding series

	Mosq. lots	Mosq.dissect.	Midgut dissections	
	No.(%pos.)	No.(%pos.)	No.(% pos.)	Range/$\bar{x}$ oocysts
Donor monkeys yielding infected mosquitoes				
Aotus trivirgatus (15 subjects)				
GML strain	86(26.7)	1,719(5.35)	1,684(3.86)	1-41/8.37
Escobal strain	86(25.6)	1,710(5.56)	1,675(3.04)	1-42/9.75
Donor monkeys not yielding infected mosquitoes				
Aotus trivirgatus (28 subjects)				
GML strain	133	2,550	2,540	-
Escobal strain	133	2,550	2,540	-
Saimiri sciureus (5 subjects)				
GML strain	12	235	235	-
Escobal strain	12	240	240	-
Ateles fusciceps (3 subjects)				
GML strain	12	235	235	-
Escobal strain	12	235	235	-

Table 17

Comparative susceptibility of Anopheles albimanus and A. aztecus to Plasmodium vivax in paired feeding trials.

Host species	No. trials (Lot pairs)	Tabulations from infected lots								
		A. albimanus					A. aztecus			
		No. lots inf.	Lot inf. rates	Pos./Tot. mosq. (%)	Oocysts Range	Oocysts $\bar{X}$	No. lots inf.	Lot inf. rates	Pos./Tot. mosq. (%)	Oocysts Range	Oocysts $\bar{X}$
Aotus trivirgatus	212	20	4-52	84/409 (20.5)	1-41	5.7	8	4-80	18/111 (16.4)	1-6	2.1
Saimiri sciureus	59	2	4-9	3/46 (6.5)	2-10	5.0	4	9-71	10/41 (24.4)	1-31	7.8
Saguinus geoffroyi	28	4	4-11	8/102 (7.8)	1-10	2.4	1	13	2/15 (13.3)	1-4	2.5
Ateles fusciceps	56	5	5-17	13/118 (11.0)	1-18	3.0	3	5-12	5/63 (7.9)	1-7	3.5
Ateles geoffroyi	22	0	-	-	-		0	-	-	-	

Table 18

Anopheline mosquito tests for susceptibility to strains of Plasmodium brasilianum according to donor system.

Malaria strain	Host	Anophelines tested negative	Anophelines infected
Cerro Nique	Ateles fusciceps	A. aztecus A. triannulatus C. bathana	A. albimanus
Cerro Quia	Ateles fusciceps	A. albimanus C. bathana	
Cerro Azul	Ateles geoffroyi*	A. albimanus	A. aztecus
Rio Chico	Ateles geoffroyi*	A. albimanus A. aztecus A. oswaldoi	
PB-A	Aotus trivirgatus	A. albimanus A. aztecus	
	Saguinus geoffroyi	A. albimanus	
	Cebus capucinus	A. albimanus	

* Naturally acquired infections.

Table 19

Anopheline mosquito tests for susceptibility to *Plasmodium simium* according to donor system.

Malaria strain	Host	Anophelines tested negative	Anophelines infected
NYU	*Aotus trivirgatus*	*A. aztecus* *A. oswaldoi* *A. punctimacula* *C. bathana*	*A. albimanus*
	Ateles fusciceps	*A. albimanus* *A. aztecus*	
	Ateles geoffroyi	*A. albimanus* *A. aztecus*	
	Saguinus geoffroyi	*A. albimanus*	
	Cebus capucinus	*A. albimanus*	
	Alouatta villosa	*A. albimanus* *A. aztecus*	

Table 20

Comparison of Plasmodium vivax (Achiote strain) transmissions in 63 experiments using Aotus trivirgatus and other species as co-recipients.

	Positive/total expts. (%)	Aotus		Other species
		Positive/total recipients (%)		
Aotus - Ateles geoffroyi	4/4(100)	1/7(14)	-	5/5(100)
Aotus - Ateles fusciceps	7/17(41)	1/30(3)	-	6/19(32)
Aotus* - Saimiri*	13/21(62)	10/73(14)	-	22/110(20)
Aotus - Saguinus	7/21(33)	2/36(6)		4/23(7)

* Intact subjects only.

Table 21

Summary of experiments to induce infections with sporozoites of Plasmodium vivax (Achiote strain) in intact and splenectomized monkeys.

	Positive/total expts. (%)	Intact	Splenectomized
		Positive/total recipients (%)	
Ateles geoffroyi	7/7(100)	5/5(100)	4/4(100)
Ateles fusciceps	12/26(46)	1/1(100)	11/29(38)
Saimiri sciureus	23/43(53)	37/187(20)	6/14(43)
Saguinus geoffroyi	6/34(18)	2/23(9)	4/21(19)
Aotus trivirgatus	21/207(10)	19/182(10)	7/63(11)

Table 22

Plasmodium vivax transmission success to methionine-treated monkeys, with comparison of infections in untreated co-recipients.*

Recipients	Transmission Parameters					Infection Parameters			
	No. inf. mosq.	Sporoz. conc. range[a]	Total plus rating	Inoc. route	Days exam.	Days prepat.	Days pat.	Parasit. max. per cmm	No. relapses
				Ateles geoffroyi					
Test 1 (subspecies A. g. vellerosus)									
Treated-7847	30	2-4+	105+	Hepatic	241	27	37	100	4
Control-7846	"	"	"	"	"	196	12	<10	0
Test 2 (subspecies A. g. panamensis)									
Treated-5639	21	2-4+	70+	"	448	47	33	<10	3
Control-7181	"	"	"	"	"	133	21	20	4
				Ateles fusciceps					
Control-6354[s]	17	3-4+	56+	IV	>450	23	21	1,800	1
Treated-6356[s]	"	"	"	"	>150	Neg.	-	-	-
Treated-6358[s]	"	"	"	"	"	"	-	-	-
				Saimiri sciureus					
Treated-4783[s]	30	1-4+	72+	IP	244	58	8	<10	0
Control-4687[s]	"	"	"	"	306	12	137	20,900	3
				Aotus trivirgatus					
Test 1									
Treated-7626[s‡]	40	1-4+	96+	IP	>119	Neg.	-	-	-
Test 2									
Treated-7639	18	2-4+	51+	"	52	"	-	-	-
Treated-7640	"	"	"	"	43	"	-	-	-
Control-7647	"	"	"	"	>105	"	-	-	-

* Treated subjects received 2 gms orally per day, days 0-30.
a) Sporozoite grouping: 1+,1-9;2+,10-99;3+,100-999;4+,>999.
s Splenectomized hosts.
‡ Companion recipient in above *Saimiri* test series.

Table 23

Summary of hepatic biopsy experiments and demonstration of *Plasmodium* exoerythrocytic stages.

Monkey no.	Alter. or Rx	Inoculum-total + rating	Prepat.pd. or (neg.days exam.)	Biopsy day-post inoc.	No. sections exam.	No.EE bodies	Mean longest diameter-μ
				P. vivax (Achiote Strain)			
Ateles geoffroyi							
5639	M[a]	70	47	7;9	871;1,906	49;7	36.2[b];58.8
7181	None[c]	70	133	7;9;209	1,108;1,377;716	~57;10;0	30.0[d];47.4[e];-
7847[f]	M	105	27	7;10	946;612	0;0	- ; -
7846[f,g]	None	105	196	7;10	551;800	0;0	- ; -
5509	S[h]	138	20	8	95	0	-
Ateles fusciceps							
328	S	105	12	7;10	176;76	1;0	25.0;-
488	S	193	22	7;10	1,731;416	10;0	18.9;-
5833	S	323	10	10;15	1,642;1,127	2;0	65.7;-
8090	S	171	(>100)	5;9	492;417	0;0	-;-
Saimiri sciureus							
6965	None	138	12	9	324	8	36.1
7005	None	45	(10)	7;10	562;485	72;~156	28.2[i];48.1[j]
5213	None	29	(117)	14;21	463;376	0;0	-;-
7994	None	77	(8)	7	412	1	18.5
8110[k]	None	39	(109)	7;10	243;232	0;0	-;-
7370	None	66	31	7;10	407;540	0;4	-;55.5

Table 23(cont'd.)

Monkey no.	Alter. or Rx	Inoculum-total + rating	Prepat.pd. or (neg.days exam.)	Biopsy day-post inoc.	No. sections exam.	No.EE bodies	Mean longest diameter-μ
Cebus capucinus							
4894	S	319	(>237)	9	233	~ 120	67.6[1]
5290	None	241	(>120)	7;10	505;521	4;1	32.2;13.8
5293	None	140	(>100)	9;21;62	758;394;425	6;0;0	57.9;-;-
4897	None	111	(>100)	7;10	427;395	0;0	-;-
Cebus apella							
67	None	144	(>253)	7;9	562;553	1;2	31.3;64.3
Aotus trivirgatus							
6127	None	138	(18)	11;18	197;438	2;0	10.7;-
6151	None	30	(>82)	8	107	0	-
Saguinus geoffroyi							
7260	None	43	(7)	7	1,622	0	-
7591	None	138	(90)	9;16	713;493	0;0	-;-
7800	None	79	(33)	7;9;33	506;565;400	0;0;0	-;-;-
7842	None	67	(43)	14;24	470;425	0;0	-;-
7913	None	101	(19)	7;9	388;427	1;0	18.5;-
7937	None	81	(10)	7;9	438;408	0;0	-;-
8117	None	162	(113)	7;10	399;515	0;0	-;-
Alouatta villosa							
8008	None	76	(>127)	7;10	478;438	0;0	-;-
8012	None	76	(48)	7;10	405;393	0;0	-;-
8014	None	93	(>120)	7;10	500;405	0;0	-;-
8013	None	151	(>120)	9	390	0	-

Table 23 (cont'd.)

Monkey no.	Alter. or Rx	Inoculum-total + rating	Prepat.pd. or (neg.days exam.)	Biopsy day-post inoc.	No. sections exam.	No.EE bodies	Mean longest diameter-μ
			P. falciparum (Guayabalito-4 Strain)				
Cebus capucinus							
5296	None	175	(>120)	8	~260	0	-
5299	None	175	(>120)	12	~200	0	-

a M= Methionine administered orally (2 gms. daily), days 0-30.
b 46 measured.
c Control for 5639.
d 24 measured.
e 4 measured.
f Guatemalan origin (*A. g. vellerosus*).
g Control for 7847.
h S= splenectomized.
i 50 measured.
j 59 measured.
k Peruvian origin.
l 50 measured.

Table 24

Plasmodium vivax exoerythrocytic stages in Ateles geoffroyi; assessment of development in methionine-treated (5639) and untreated control (7181) subjects.

Biopsy day	Host no.	No. bodies measured	Range (Mean ± SD ‡) μ Length	Width	L/W Ratio
7	5639	46	29.6-48.1(36.2±4.75)	22.2-40.7(29.3±4.43)	1.0-1.57(1.25±0.18)
	7181	24	22.2-37.0(30.0±4.06)	18.5-33.3(26.1±4.52)	1.0-1.6(1.19±0.16)
Significance of difference			t= 4.696 **	t= 2.806 *	t= 1.364
9	5639	7	48.1-70.3(58.8±8.30)	33.3-51.8(40.1±8.39)	1.14-2.11(1.52±0.38)
	7181	4	33.3-48.8(47.4)	25.9-43.8(36.9)	1.11-1.60(1.30)

‡ Calculated if 5 or more stages found.

** Significant at 0.001 level.

* Significant at 0.01 level.

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