19

Plasmodium brasilianum Gonder and von Berenberg-Gossler , 1908

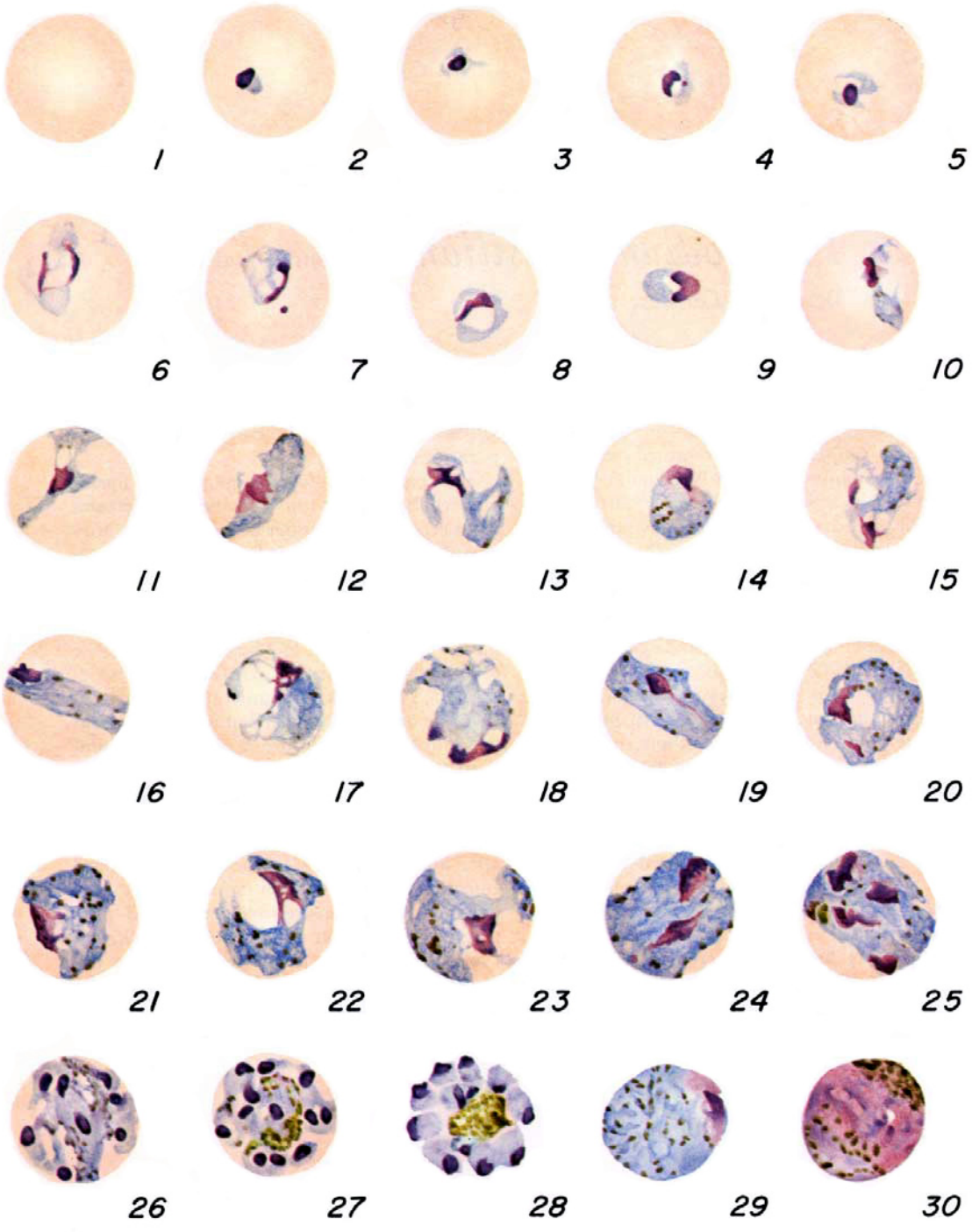
IN 1908, Gonder and von Berenberg-Gossler had the opportunity to examine the blood of a Cacajao monkey, *Brachyurus calvus* (= *Cacajao calvus)* imported to Hamburg, Germany, from the Amazon region of Brazil. In it, they encountered a quartan-like malaria parasite to which they gave the name *Plasmodium brasilianum.* The following year (1909), von Berenberg-Gossler made a careful study of the parasite and compared it with *P. malariae,* which it closely resembles, and other malarias then known from monkeys.

Seidelin (1912) had kept a spider monkey *(Ateles* sp.) in his laboratory in Merida, Yucatan for some time and in its blood, he found delicate rings which some authors, including Wenyon (1926), considered to be a plasmodium. Repeated examination of the blood showed only rings with one or two chromatin masses which is emphasized on his colored plate, and, therefore, as suggested by Garnham (1966), it was probably a piroplasm.

Plasmodium brasilianum is relatively common in the monkeys of Panama where Clark (1930, 1931) first encountered it. Then, the Taliaferros (1934, a, b, and 1944) carried out an exhaustive experimental study of the parasite. Porter, *et al,* (1966) summarized the records involving 1994 primates from Panama, collected from 1931 through 1957, among which 4 species in 3 genera exhibited the parasite.

The parasite is fairly common in northwestern Brazil. In 1969, Deane and Ferreira Neto reported *P. brasilianum* from the territory of Amapa, also known as Brazilian Guyana, in eastern Amazonia. There is only one report of a natural infection in Venezuela (Serrano, 1967) but numerous surveys in Colombia, the most recent was that of Marinkelle and Grose (1968), have shown the parasite to be widespread. The work of Dunn and Lambrecht (1963) extended the distribution of this parasite into the lowland forests of Colombia and Peru.

Following the work of the Taliaferros in Panama, little attention was given to the malarias of the New World monkeys until after the National Institute of Allergy and Infectious Disease's group launched an extensive program following accidental and purposeful infections in man with *P. cynomolgi* in 1960. Beginning in 1964, Deane and his co-workers entered upon an overall study of the simian malarias of Brazil which has been extremely fruitful.



G. H. Nicholson

PLASMODIUM BRASILIANUM

Cycle in the Blood

PLATE XXXVI

The initial forms in the peripheral blood are small rings with a prominent dark-staining nucleus. As growth proceeds, a small vacuole is formed which eventually disappears in the older forms. The rings often exhibit accessory dots. The nucleus may elongate to form a part of the periphery of the older rings (Figs. 6, 7). With the disappearance of the vacuole, the compact parasite displays irregular protrusions (Figs. 10, 11). These loitering amoeboid forms are not unlike similar ones seen in *P. malariae.* At about this time black granular pigment appears scattered in the blue-staining cytoplasm. Band forms appear during the late trophozoite stage (Figs. 16, 19) and although these forms are found in the blood of all simian species susceptible to the infection, they occur with greater frequency in *Alouatta.* The host cell is not enlarged and does not appear inconvenienced by the parasite. At about 50 hours, the parasite occupies a large part of the erythrocyte, at which time, it begins to divide. The process of schizogony moves toward completion generally with decided synchronicity. At maturity, each schizont harbors 8 to 12 merozoites but there may be only 4 or up to 16 arranged more or less as a rosette or, what is sometimes described, as a "daisy-head" (Fig. 28). The mature schizont may produce a slight increase in the size of the host cell; a condition not found with *P. inui.* With appropriate staining, Ziemann's stippling can be demonstrated during the late ring stage.

The young gametocytes are difficult to recognize and, as is common in all quartan infections, sexual forms are scarce at best. The mature forms fill the host cell and cause some enlargement of it. The macrogametocyte, which stains a median blue, harbors a compact pink-staining nucleus with a darker reddish area and occupies an eccentric position. The dark pigment, in the form of short rods, is scattered in the cytoplasm (Fig. 29). The microgametocyte exhibits a large diffuse nucleus which stains dark pink. The cytoplasm takes a very pale blue to purplish stain, with the pigment in large granules more or less scattered in the matrix.

The asexual cycle requires 72 hours which the Taliaferros described and illustrated in precise detail. They went on to show (1934) that the decidedly synchronous cycle could be reversed in a short time if the infected animals were subjected to a reversal of night and day conditions. Under normal conditions sporulation took place around 8 a.m. but after the conditions were reversed the cycle slowly became modified so that it occurred at 8 p.m. Some years later (1940) Young, Coatney and Stubbs produced the same results with *P. malariae* in man.

Sporogonic Cycle

PLATE XXXVII

Clark and Dunn (1931) reported development of *P. brasilianum* to the sporozoite level in *Anopheles tarsimaculatus* (= *aguasalis),* and *A. albimanus.* Garnham *et al* (1963) infected *A. aztecus, A. atroparvus,* and *A. albimanus.* At an incubation temperature of 26 to 28° C, the oocysts grew slowly and were characterized by a fragile cyst wall. On the 8th day oocysts measured 20 to 24 μ and contained pigment arranged along irregular threads, in circles, or in clusters. By the 12th day, the oocysts approached maturity with diameters of 38 to 45 μ. Sporozoites were present in the salivary glands on day 13. The sporozoites were reported to be sickle-shaped with a central nucleus. In the

PLATE XXXVI.—*Plasmodium brasilianum*.

Fig. 1. Normal red cell.
Figs. 2-5. Young trophozoites.
Figs. 6-13. Growing trophozoites.
Figs. 14-23. Nearly mature and mature trophozoites (Note band-forms Figs. 16, 19)
Figs. 24-26. Developing schizonts.
Figs. 27-28. Mature schizonts.
Fig. 29. Mature macrogametocyte.
Fig. 30. Mature microgametocyte.

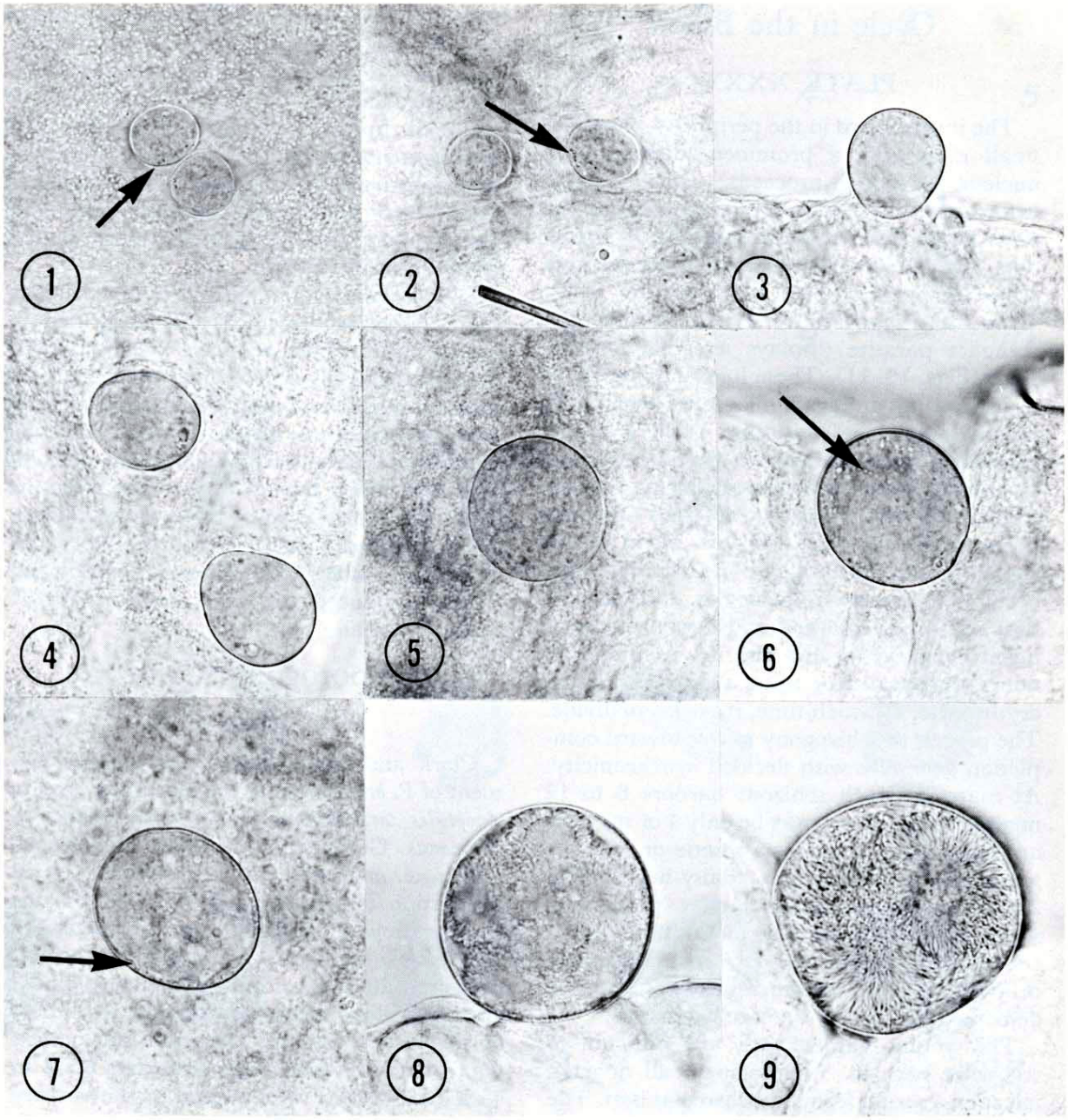


PLATE XXXVII.—Developing oocysts of *Plasmodium brasilianum* in *Anopheles freeborni* mosquitoes. X 580.

Fig. 1. 8-day oocysts with small grains of pigment.
Fig. 2. 9-day oocysts showing pigment near periphery.
Fig. 3. 10-day oocyst.
Fig. 4. 11-day oocysts.
Fig. 5. 12-day oocyst.
Fig. 6. 13-day oocyst showing small number of vacuoles.
Fig. 7. 1-day oocyst showing earliest stage of differentiation.
Fig. 8. 15-day differentiating oocyst.
Fig. 9. 18-day fully differentiated oocyst.

fresh state, they measured about 14 to 16 μ in length.

Garnham *et al* (1963) studied the fine structure of the sporozoites of *P. brasilianum*. Their material was not of the best quality and therefore they were unable to give a complete and precise description. They found that the pellicle was about 30 Mμ in width and appeared as two layers separated by a less dense area. An apical cup was not demonstrated but a well-defined micropyle was seen. Peripheral fibers were hollow, but their exact number could not be determined. The "paired organelle" was an obvious structure suggesting a secretory function.

In our studies, *A. freeborni* has proved to be the most suitable mosquito for observing the sporogonic cycle of *P. brasilianum* (Table 29). At an incubation temperature of 25° C, on day 6, the mean oocyst diameter was 11 μ, with a range of 9 to 13 μ. The oocysts continued to grow so that by day 14, the mean size was 40 μ, with a range of 17 to 60 μ. At this time, the first signs of differentiation were apparent. The development was slow, however, and sporozoites were not present in the salivary glands until 17 days after feeding.

TABLE 29.—Oocyst diameters of *Plasmodium brasilianum* in *Anopheles freeborni* and *A. stephensi.*

Days after Infection	*A. freeborni*			*A. stephensi*		
	No.	Range	Mean*	No.	Range	Mean
6	43	9-13	11			
7	176	8-18	13			
8	148	11-20	15			
9	181	12-25	18	24	14-24	19
10	181	12-32	21	23	15-28	23
11	183	14-39	25	13	28-37	30
12	123	14-42	30			
13	142	17-53	34			
14	196	17-60	40†	16	28-51	38†
15	175	20-63	41†			
16	150	17-70	46†			
17	112	26-70	53†**			
Totals	1810	9-70		76	14-51	

* Measurements expressed in microns; incubation temperature of 25° C.
† Oocyst differentiation.
** Sporozoites present in the salivary glands.

A few measurements of oocysts developing in *A. stephensi* mosquitoes gave values well within the range of the diameters seen in the

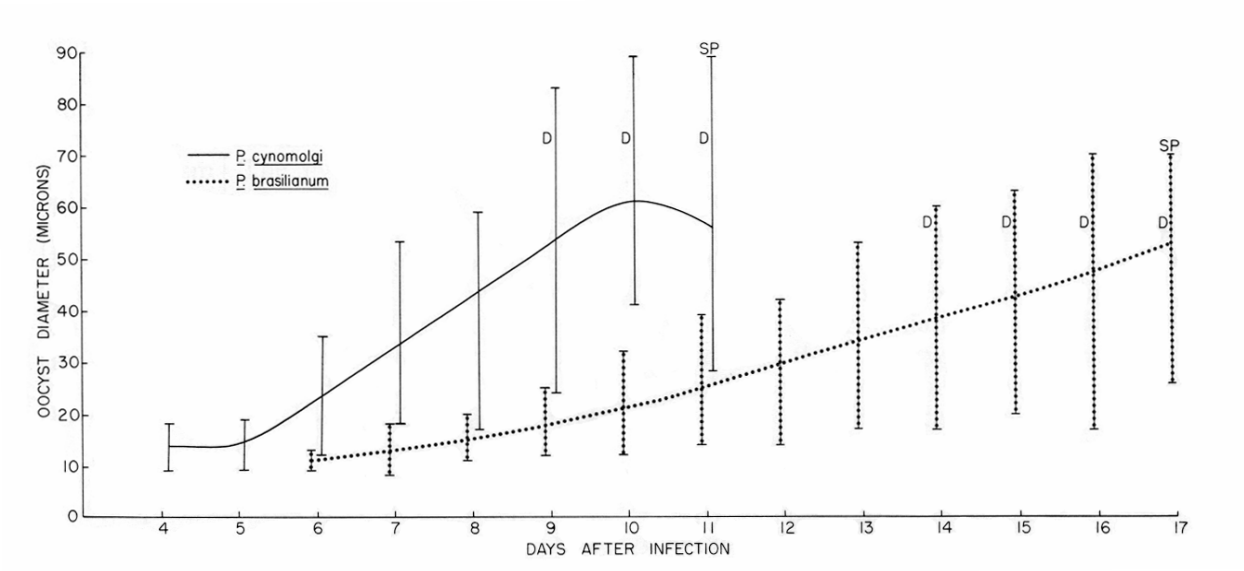


FIGURE 50.—Range in oocyst diameters and the mean oocyst diameter curves of *Plasmodium cynomolgi* and *P. brasilianum* in *Anopheles freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

A. freeborni, none of the mosquitoes of this species survived long enough to determine if sporozoites would invade the salivary glands, Other species which have developed sporozoites in the salivary glands were *A. maculatus* and rarely, *A. quadrimaculatus* (Collins *et al,* 1969).

If a comparison is made of the growth rate of *P. brasilianum* and *P. cynomolgi* (Fig. 50), one sees a marked difference between the two parasites. The mean oocyst diameter of *P. cynomolgi* at day 8 is about the same as that of *P. brasilianum* at day 15. The maximum oocyst diameters of *P. cynomolgi* far exceed those of *P. brasilianum* and the time required for completion of the life cycle, i. e., the appearance of sporozoites in the salivary glands, was shorter by 6 days. A comparison of the oocyst growth rates of the two quartan parasites, *P. malariae* and *P. brasilianum,* (Fig. 51) indicates that the mean oocyst diameters of these two parasites in *A. freeborni* mosquitoes are almost identical. Such a close similarity in oocyst growth rate curves, was not found between any of the other human and simian malaria parasites. From a study of the sporogonic growth rate, it is strongly suggestive, that these parasites, *P. brasilianum* and *P. malariae,* are almost identical.

Sporozoites of *P. brasilianum* in *A. aztecus* were shown to be infective (Garnham *et al,* 1963) in that a strain from the squirrel monkey *(S. sciureus)* was passaged, by the combination of mosquito feeding and intravenous inoculation of dissected salivary glands, into another squirrel monkey and also into a *Cebus capucinus* monkey. In our studies, one *Ateles paniscus* monkey was infected through bites of *A. freeborni* mosquitoes, and six monkeys (2 *A. paniscus,* 3 *S. sciureus,* and 1 *Aotus trivirgatus)* were infected by the intravenous or intrahepatic inoculation of infected salivary glands. The prepatent periods in these animals ranged from 18 to 43 days with a mean of 33.1 days.

Cycle in the Tissue

PLATE XXXVIII

The first workers to study the EE bodies of *P. brasilianum* were Garnham *et al* (1963). They made two unsuccessful attempts to find the tissue stages but were rewarded on the third try when they located a single 11-day old EE body after examining some 500 sections. They described the parasite as oval, 20 by 38 μ, and located in a parenchyma cell of the liver possessing two enlarged nuclei. The parasite was

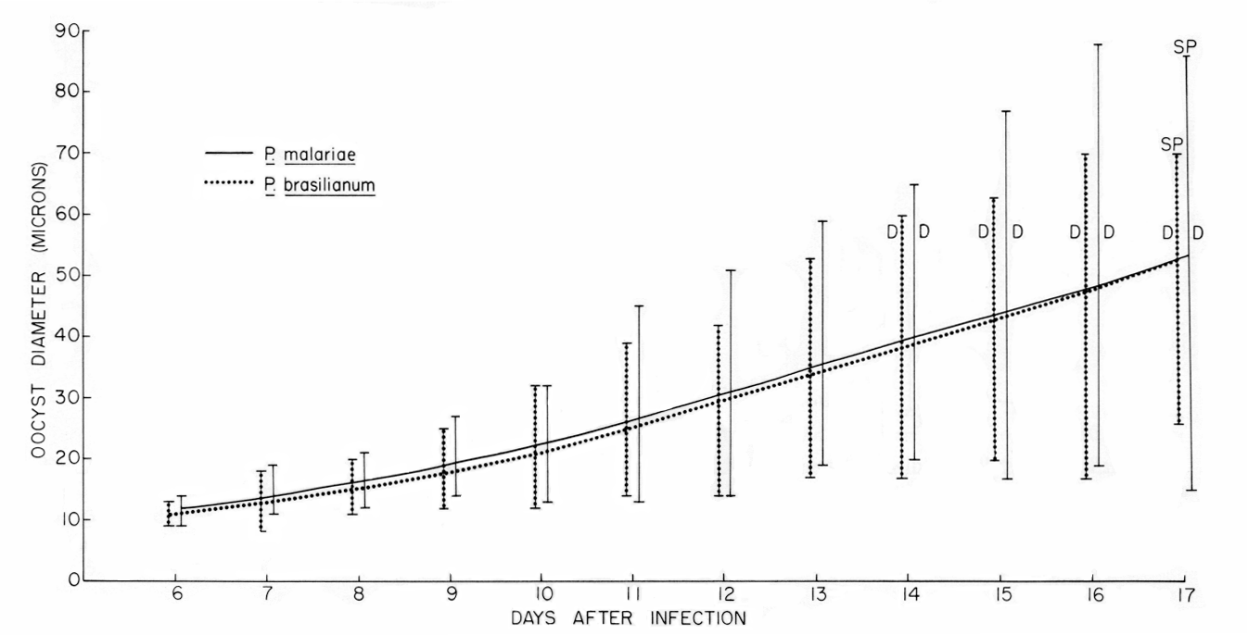


FIGURE 51.—Range in oocyst diameters and the mean oocyst diameter curves of *Plasmodium malariae* and *P. brasilianum* in *Anopheles freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

enclosed within a definite membrane which was decidedly convoluted or scalloped. Small peripheral vacuoles were present, filled with a pink-staining substance. The cytoplasm was granular and tenuous, and exhibited vague cleavage lines. The nuclei of the schizont were large and arranged in a cluster of irregular dots measuring 1 to 1.5 μ. They estimated that the schizont was a day or so from maturity indicating a primary cycle of some twelve days.

In 1969, Sodeman *et al* described the EE stages of a strain of *P. brasilianum* which, like Garnham's strain, had been isolated from a naturally infected squirrel monkey, *Saimiri sciureus.* They were unable to locate the parasites in biopsy material taken at 7 days, but found them in tissue taken at 14, 18, and 21 days after sporozoites were introduced. These were illustrated in a series of four well-executed plates.

The 14-day EE bodies (Figs. 1, 2) were elliptical while those of the 18th (Fig. 3) and 21st days (Figs. 4-6) showed a tendency toward lobulation. A distinct cell membrane was evident surrounding the EE bodies. The edges of the bodies were smooth except in situations where shrinkage, probably due to fixation, produced a scalloped effect. The nuclei of the EE bodies were irregular in shape, stained magenta, varied from 0.5 to 1.5 μ in diameter, and were distributed evenly through the cytoplasm. The cytoplasm stained pale blue and was granular in texture. Irregular-shaped, dark blue, aggregated flocculi (Fig. 3) were present in all the stages, though not found in every EE body; they were least prominent in the 21-day bodies. The parasitized liver cells commonly exhibited enlarged double nuclei (Fig. 5).

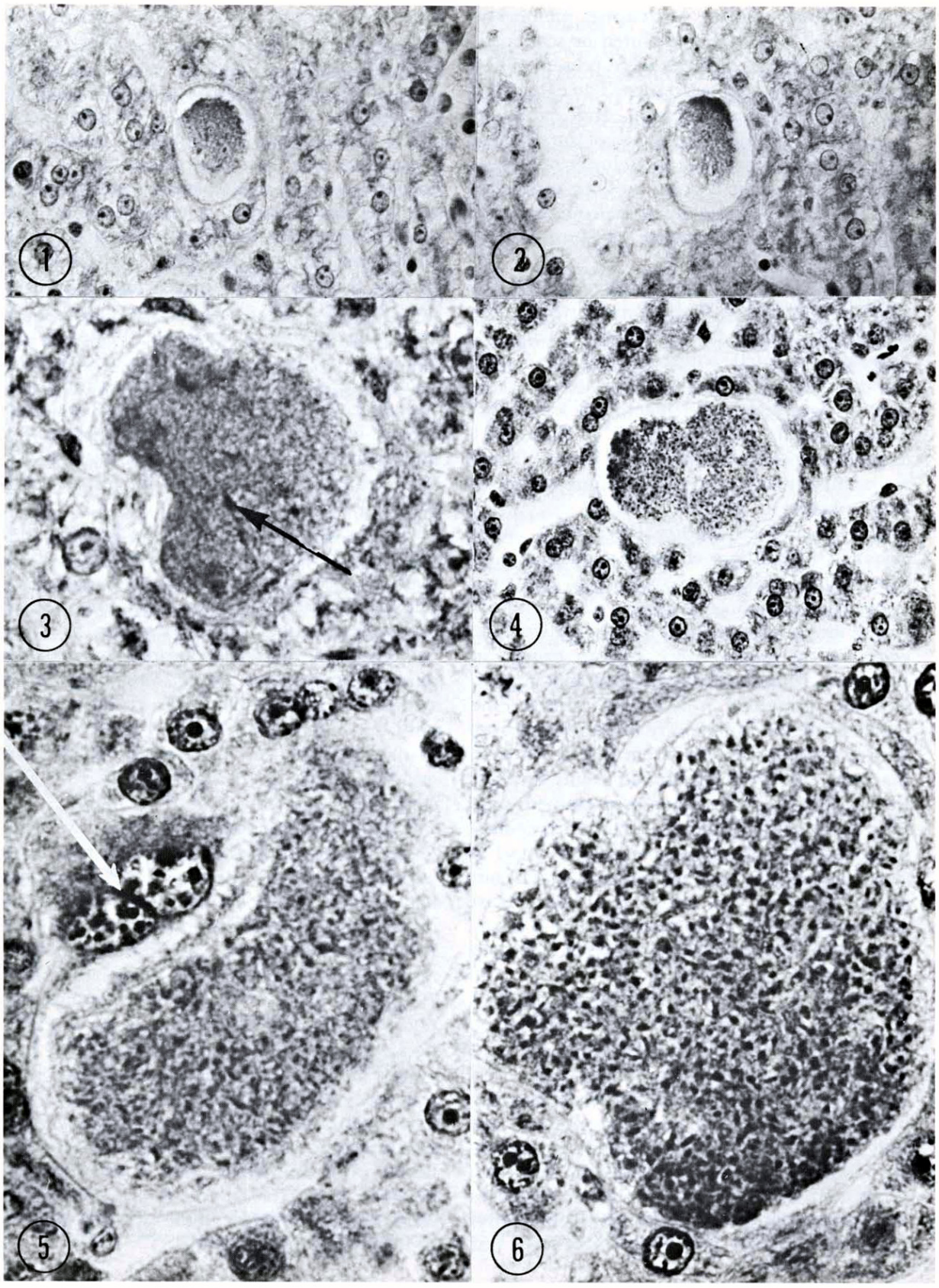
Among the special morphological features of the *P. brasilianum* EE bodies were the presence of needle-like clefts in the 14- and 21-day material. In many cases, these clefts were outlined with basophilic material, and basophilic strands. The authors pointed out that the lack of clefts and strands in the 18-day bodies may indicate individual variation prominent in EE bodies of the same species along with the rarity of the forms for study. Garnham *et al* (loc. cit.) mentioned clefts but described them as vague. Clefts have been reported in the EE bodies of other simian malarias and in *P. malariae* of man. Lupascu *et al* (1967) reported them in the late stages of *P. malariae.* In *P. brasilianum,* the clefts were not as prominent as represented in *P. malariae.*

Lupascu *et al* also reported strands in their 15-day material of *P. malariae* which stained red and were limited to that one stage. In *P. brasilianum,* on the other hand, the strands were basophilic and present in the 14-day and 21-day sections; such strands have not been described for the other simian malarias. A prominent feature of the 21-day stage was the retraction of the cytoplasm from the limiting membrane, sometimes associated with delicate vacuoles. Garnham *et al* described a similar feature in his 11-day stage, and Lupascu *et al* for the late stage of *P. malariae.* Bray (1957) found the same condition in his *P. ovale* material.

Greatly enlarged host cell nuclei were also found in *P. brasilianum* by Garnham and in liver cells housing *P. malariae* and *P. ovale.* This finding has not been described for any EE bodies of the other simian malarias.

Considering the slow rate of growth of the parasite, the size of the *P. brasilianum* tissue schizont is remarkable. The 21-day stage is only 6 μ larger than the average size of the 11-day parasite of *P. cynomolgi. Plasmodium malariae* in man at 14 days averaged 47 μ as compared with 30.6 μ for *P. brasilianum.*

Further comparisons were made by Sodeman *et al,* but suffice it to say here that those authors were of the opinion that *P. brasilianum* had certain features which *might* distinguish it from the other simian malarias, including *P. inui,* and that there was close morphologic resemblance to the human malarias, especially *P. malariae.* The presence of enlarged host cell nuclei, needle-like clefts, and peripheral retraction with vacuoles are features not seen previously in simian malaria material. However, they are all present in the human malarias. They mentioned also, that there was greater morphologic relationship between the EE bodies of *P. brasilianum* and *P. malariae* than between *P. brasilianum* and *P. inui.* Additional relationships between these species will be dealt with in later sections.


1
2
3
4
5
6

Course of Infection

Taliaferro and Taliaferro (1934) showed that blood-induced infections with *P. brasilianum* were characterized by an initial rise in the number of parasites, a marked dimunition in numbers, a low grade blood infection, and finally, short periods of subpatent parasitemia interspersed with spontaneous recrudescences. The height to which the parasitemia rose varied among individuals of the same species, but showed a tendency to be more acute among the white-throated *(Cebus capucinus)* and spider monkeys *(Ateles geoffroyi)* than among the howlers *(Alouatta palliata* (= *villosa)*. Sharp peaks in temperature occurred at sporulation, particularly in the spider, howler, and night monkeys *(Aotus zonalis* (= *trivirgatus)* and in the marmoset *(Leontocebus* (= *Saguinus) geoffroyi),* provided parasites were in sufficient numbers.

In our studies, infections have been obtained in *Saimiri sciureus, Ateles paniscus,* and *Aotus trivirgatus* monkeys both by blood inoculation and by the introduction of sporozoites. In intact *A. paniscus* monkeys (Fig. 52), the parasitemia rose slowly to a peak of approximately 10,000 per mm^3 by day 16, followed by a fall, and then a secondary rise to a level almost equal to the initial peak by day 60. In the intact *S. sciureus* monkeys, the parasitemia curve exhibited a saddle-back effect with two peaks, on days 8 and 20, followed by a slow decline in the parasitemia. In *A. paniscus,* the median parasitemia by day 60 was 10,000 per mm^3, whereas in the *S. sciureus,* the median parasitemia, on day 60, was approximately 100. In splenectomized *S. sciureus* monkeys, the peak

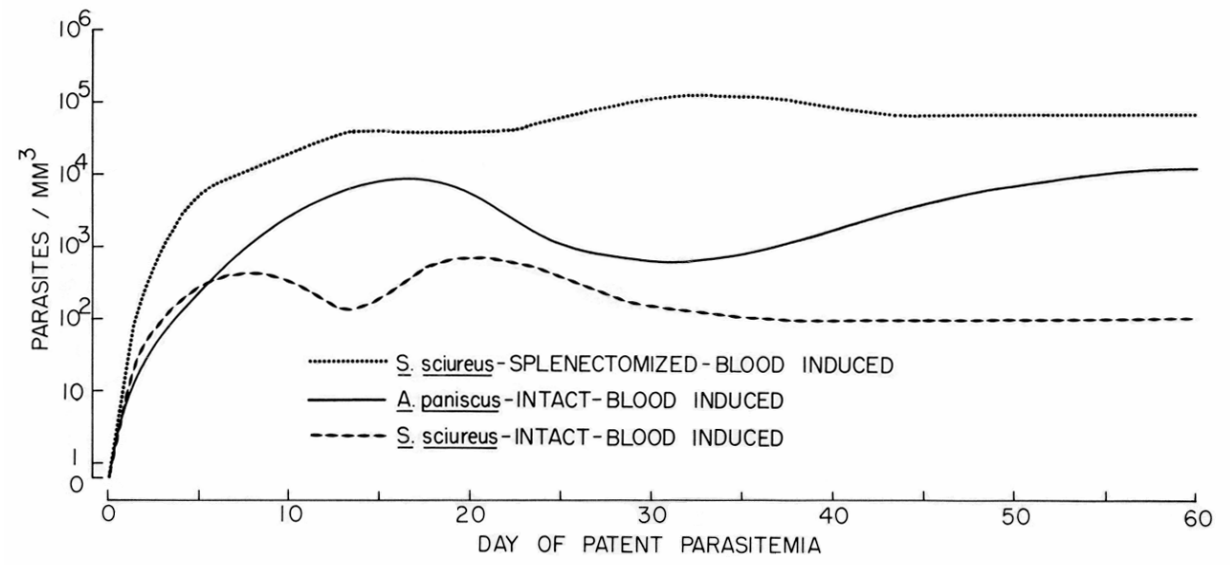


Figure 52.—Median parasitemia curves of *Plasmodium brasilianum* in 13 intact *Ateles paniscus*, 7 intact *Saimiri sciureus*, and 12 splenectomized *S. sciureus* monkeys inoculated with parasitized blood.

PLATE XXXVIII.—Exoerythrocytic bodies of *Plasmodium brasilianum* in liver tissue of the squirrel monkey, *Saimiri sciureus*.

Fig. 1. 14-day body showing elliptical shape. X 580.
Fig. 2. 14-day body. X 580.
Fig. 3. 18-day body showing dark, irregular-shaped, aggregated flocculi. X 1450
Fig. 4. 21-day body. X 580.
Fig. 5. 21-day body showing two enlarged host cell nuclei. X 1450.
Fig. 6. 21-day body showing irregular-shaped parasite nuclei. X 1450.

parasitemia was reached after about 35 days. At 60 days, the median count was approximately 70,000 per mm^3. None of the animals, whether intact or splenectomized, died or required chemotherapy for survival. One splenectomized *A. trivirgatus* monkey, infected by the inoculation of sporozoites, had a peak parasitemia of 35,600 per mm^3 on day 35 after which, the parasitemia slowly declined to a subpatent level by day 90. No further parasites were seen in the blood of the animal during the next 57 days, when it died.

In our experience, infections with *P. brasilianum* in *Ateles* and *Saimiri* monkeys usually persist for an extended period of time, during which the presence of gametocytes allows for the infection of mosquitoes, and such infectivity can continue for at least 249 days. On the other hand, daily feedings on an *A. trivirgatus* monkey, infected with *P. brasilianum,* resulted in no infections; an unexpected occurrance in view of our other successes.

During the course of an infection, the parasitemia may rise and fall in what, at times, appears to be a predetermined pattern. This was observed in an *A. paniscus* monkey (AT-36) inoculated with parasitized blood. The animal had a low-grade infection for approximately 7 weeks at which time it was splenectomized. The parasitemia rose to a maximum of 120,500 per mm^3 one month later and then slowly dropped to a near negative level after six weeks. This was followed by a subsequent rise to a maximum count of 52,000 per mm^3 followed by a subsequent drop to zero. Following the initial parasitemia, 6 additional periods of high parasitemia occurred, followed by drops to negative or near negative levels. The intervals between these peaks were 3.5, 3, 4, 5, 4, and 4 months, respectively, after which the animal failed to exhibit parasites during an observation period of 18 months. Because the animal was blood-inoculated, the appearance of pronounced waves of parasitemia following periods of latency were true recrudescences. The observations in this animal suggest an almost set time interval for the development of new antigenic variants--about four months. It would appear that the animal had then been able to produce an immunity to all the variants which the infection could produce thereby preventing any visible reoccurrence of the infection.

The first investigators to carry out experiments toward infecting man with *P. brasilianum* were Clark and Dunn (1931, 1931a). These investigators selected 8 volunteers who had recently arrived in Panama from their home states in the U. S. where malaria was not endemic. Five of the volunteers and a control monkey were given parasitized blood intravenously from a black spider monkey. Two other volunteers plus each of the original five and a control animal received parasitized blood subcutaneously also from a black spider monkey. The control monkeys developed the infection but only one of the volunteers gave any evidence of infection which was recorded as a few doubtful intracorpuscular bodies on an occasional blood film. After two weeks of observation the authors considered the trial a failure.

At that juncture, they decided to attempt infection by mosquito bite. They were able to infect *A. albimanus,* and *A. tarsimaculatus* which were allowed to bite seven volunteers. The men were observed for varying lengths of time, but again, only one exhibited any evidence of infection which consisted of several elevations of temperature at or near 100° F. and, on two occasions, "one or two forms which we consider to be malarial parasites but there was nothing satisfactory to report." It is unlikely, but possible, that a *P. brasilianum* infection was initiated in any of the volunteers.

Our own studies toward infecting man with *P. brasilianum* actually began in London. The senior author (GRC) had stopped there in 1962 to visit Professor P. C. C. Garnham at the London School of Hygiene and Tropical Medicine. In the course of the conversations, he mentioned our good fortune in getting *P. cynomolgi* to grow in man and offered the prediction that it probably would be extremely difficult, if not impossible, to get other species to infect man. I thought otherwise, and offered a wager that we could infect man with *P. brasilianum* in a matter of weeks. Upon my return to Washington, the problem was--where to obtain *P. brasilianum?* Dr. Carl Johnson, then Director of the Gorgas Memorial Laboratory in

Panama, had some 8 months before agreed to be on the lookout for a brasilianum infected monkey, but we had had no word from him. Knowing Dr. Carl's penchant for not writing letters, a young staff member was dispatched to Panama with instructions to remain there until he had obtained a monkey infected with *P. brasilianum.* He departed for Panama and four days later called one of us (PGC) from Miami, Florida, with the query "what do I do with the infected monkey". The secret of the young man's phenomenal success in obtaining an infected monkey so readily was that Dr. Johnson had been holding the animal for some time and welcomed the opportunity to get it to us. Once the monkey, a spider *(A. paniscus*) (originally reported incorrectly as *A. geoffroyi*) was ensconced in the laboratory in Chamblee, Georgia, *A. freeborni* mosquitoes were allowed to feed on it; they became infected and were allowed to bite 9 volunteers. Five of the nine became infected (3 Caucasians and 2 Negroes) with prepatent periods of 29 to 64 days (Contacos *et al,* 1963). Later, two additional men were infected via mosquito bites with this same isolate of *P. brasilianum.* In another experiment, *A. freeborni* mosquitoes were also infected after feeding on a squirrel monkey *(S. sciureus)* naturally infected with *P. brasilianum.* This strain or isolate was subsequently transmitted by their bites to one of 3 volunteers whose prepatent period was 63 days. All together, we have been able to study brasilianum infections in 25 volunteers, of which 18 were induced by the intravenous inoculation of parasitized homologous blood. Because there was little difference between the parasitologic and clinical picture in these infections, their parasite counts and median parasitemia curve have been combined and are shown graphically in figure 53. The parasite counts rarely exceeded 50 per mm^3. The maximum count was 200 per mm^3. The duration of parasitemia did not exceed 27 days. The clinical manifestations were generally milder than seen in *P. cynomolgi* or *P. knowlesi* infections; symptoms consisted mainly of headache and loss of appetite. Fevers were present, the maximum being 103.8° F. The quartan fever pattern was hardly the rule, but appeared more consistently in the sporozoite induced infections than in those induced by blood inoculation. It did not appear, in this small number of cases, that the parasitologic or clinical picture was enhanced by blood passage

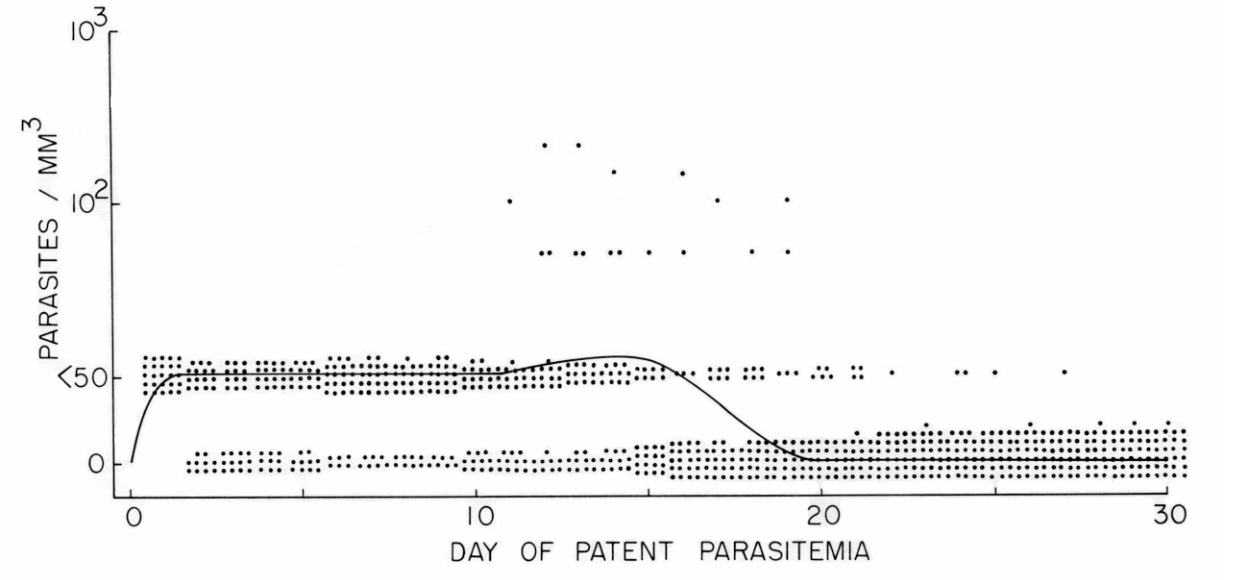


Figure 53.—Parasite counts and median parasitemia curve of 25 infections of *Plasmodium brasilianum* in man (7 sporozoite- and 18 blood-induced).

from volunteer to volunteer. When the parasite was blood-passaged back to the monkey, typical infections resulted.

This was the second simian malaria, in point of time, experimentally transmitted to man by mosquito bite and the ease with which human infections were obtained points up its zoonotic and/or anthroponotic potential when man introduces himself into a simian environment. Also, there is another point which bears discussion and that is the true identity of the brasilianum parasite. In the preceeding sections we have called attention to the close parallelism between *P. malariae* and *P. brasilianum* to the point where only an expert might presume to tell the difference either in the blood, fixed tissue, or the sporogonic cycle. This being the case, one wonders if *P. brasilianum* isn't actually a strain of *P. malariae* which became adapted to New World monkeys sometime after the early sixteen hundreds.

Host Specificity

As mentioned earlier, *P. brasilianum* has been found in Panama, Venezuela, Colombia, Peru, and Brazil. A list of the animals found infected in nature through 1969, is given in Table 30.

Although marmosets have not been found infected in nature Deane and his coworkers (1969) were able to infect *Callithrix jacchus* by transfer of parasitized blood from *Ateles paniscus*. They also transferred the infection from *A. fusca* to *Lagothrix lagotricha* and to *Saimiri sciureus.* The Taliaferros (1934) transferred the infection from *Aotus zonalis* (= *trivirgatus),* the night monkey, to *Leontocebus* (= *Saguinus) geoffroyi,* a marmoset, as well as from *Ateles darisensis* (= *paniscus)* , the black spider monkey, to *Alouatta p. palliata* (= *villosa),* the mantled howler .

Clark and Dunn (1931) showed that *Anopheles tarsimaculatus* (= *aguasalis)* and, *A. albimanus* were susceptible to infection with *P. brasilianum.* Garnham *et al* (1963) were able to infect *A. aztecus* and *A. atroparvus.* In our studies, *A. freeborni, A. stephensi, A. sundaicus, A. quadrimaculatus, A. b. balabacensis,* and *A. maculatus* have been found infectible. The relative susceptibility to infection varied from

TABLE 30.—Natural infections of *Plasmodium brasilianum* reported in Neotropical Primates from Panama, Colombia, Venezuela, Peru, and Brazil.

HOST SPECIES	REFERENCE
Alouatta fusca	Deane *et al,* 1969
Alouatta palliata	Clark, 1931
Alouatta seniculus straminea	Serrano, 1967 Deane *et al,* 1969
Alouatta villosa	Porter *et al,* 1966 Galindo*
Ateles fusciceps	Clark,1931 Porter *et al,* 1966
Ateles geoffroyi	Clark, 1931 Porter *et al,* 1966 Marinkelle and Grose, 1968
Ateles g. geoffroyi	Galindo*
Ateles g. grisestens	Galindo*
Ateles panistus (includes *"A variegates"*)	Dunn and Lambrecht, 1963
Ateles p. paniscus	Deane *et al,* 1969
Ateles p. chamek	Deane *et al,* 1969
Brathyteles arachnoides	Deane *et al,* 1969
Callicebus moloch ornatus	Renjifo and Peidrahita, 1949**
Callicebus torquatus	Deane *et al,* 1969
Cebus albifrons	Dunn and Lambrecht, 1963 Marinkelle and Grose, 1968
Cebus apella (probable infection)	Dunn and Lambrecht, 1963
Cebus apella	Marinkelle and Grose, 1968 Deane *et al,* 1969
Cebus tapucinus	Porter *et al,* 1966, Marinkelle and Grose, 1968
Cebus c. tapucinus	Clark, 1931
Cebus c. imitator	Clark, 1931 Galindo*
Chiropotes chiropotes	Deane *et al,* 1969
Lagothrix cana	Deane *et at,* 1969
Lagothrix infumata	Dunn and Lambrecht, 1963
Lagothrix lagotricha	Deane *et al,* 1969 Garnham *et al,* 1963 Marinkelle and Grose, 1968
Saimiri boliviense	Dunn and Lambrecht, 1963
Saimiri sciureus	Renjifo *et al,* 1952 Garnham *et al,* 1963 Dunn and Lambrecht, 1963 Roca-Garcia (not published) Marinkelle and Grose, 1968 Deane *et al,* 1969 Groot†

* According to Dunn and Lambrecht, 1963
** According to Marinkelle and Grose, 1968
† According to Garnham, 1966

one species to another (Table 31). By far, the most susceptible species was *A. freeborni.* No infections were obtained with *A. albimanus.*

Immunity and Antigenic Relationships

Taliaferro and Taliaferro (1934a) found that immunity to superinfection was demonstrated by a rapid decrease and disappearance of parasites from the blood of monkeys whose infection was latent, when injected intravenously with large numbers of the same strain or combination of strains. This immunity was effective immediately after the initial infection had abated and lasted for more than a year. They also showed, that there was no significant passive immunity, i.e., that serum from monkeys with latent infections with *P. brasilianum,* was without protective action when injected into uninfected monkeys, subsequently exposed to infection.

Taliaferro and Cannon (1936) stated that immunity to *P. brasilianum,* whether as a manifestation of recovery from initial infection or as immunity to superinfection, depended primarily upon the greatly increased rate of phagocytosis. The process of phagocytosis is accomplished by the macrophages of the spleen, and in a descending extent, by those in the liver and bone marrow. The Taliaferros (1944) interpreted the host's response in terms of immunity as 1) *natural immunity* associated with marked death of the parasites which may be associated with a sporadic variation in asexual reproduction and, 2) *acquired immunity* in conjunction with natural immunity which results in a heightened death rate of the parasites. The marked lowering of the asexual reproduction is due to a derangement of the asexual reproduction during crisis, a lengthening of the cycle in some parasites, and a decrease in the number of merozoites in the segmenters. Defense against *P. brasilianum* thus involves a suppression of the infection, a replacement of the erythrocytes lost by schizogony and erythrophagocytosis. Acquired immunity takes time to develop, but once acquired, responds immediately (Taliaferro and Cannon, 1936). After superinfection with large numbers of parasites, active concentration and phagocytosis of the parasites begin within one hour instead of waiting for from one to several weeks.

Antisera to *P. brasilianum* gave only a low level fluorescent antibody cross-reaction to *P. fieldi* antigen (mean reciprocal titer ratio of 100:24) and much lower levels to other primate malaria antigens (Collins *et al,* 1966a). In the reverse procedure, *P. brasilianum* antigen reacted highest to *P. inui* antisera and at a lower level to *P. cynomolgi* and *P. knowlesi* (mean reciprocal titer ratios of 100:57, 100:31, and 100:24, respectively). In many cases, antisera of *P. malariae* reacted with the *P. brasilianum* antigen at a higher level than to the homologous antigen (Collins *et al,* 1966); the reaction to antisera of *P. falciparum* was at a much lower level to the *P. brasilianum* than to the homologous antigen.

TABLE 31.—Comparative infectivity of *Plasmodium brasilianum* to seven species of *Anopheles.*

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
F-1						100
F-1 : St-1	14	110	148	60.9	16.2	12.9
F-1 : Sund	3	32	44	71.9	9.1	6.0
F-1 : Q-1	18	237	274	51.9	22.6	4.2
F-1 : Bal	14	263	201	51.0	6.0	3.2
F-1 : Mac	25	221	342	61.1	7.0	2.1
F-1 : Alb	10	182	192	50.0	0.0	0.0

* F-1 = *Anopheles freeborni,* St-1 = *A. stephensi,* Sund = *A. sundaicus,* Q-1 = *A. quadrimaculatus,* Bal = *A. b. balabacensis,* Mac = *A. maculatus,* Alb = *A. albimanus.*

** GII = Gut Infection Index = average number of oocysts per 100 guts; the GII ratio is the relationship of the GII of *A. freeborni* to another species where the GII of *A. freeborni* = 100.

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