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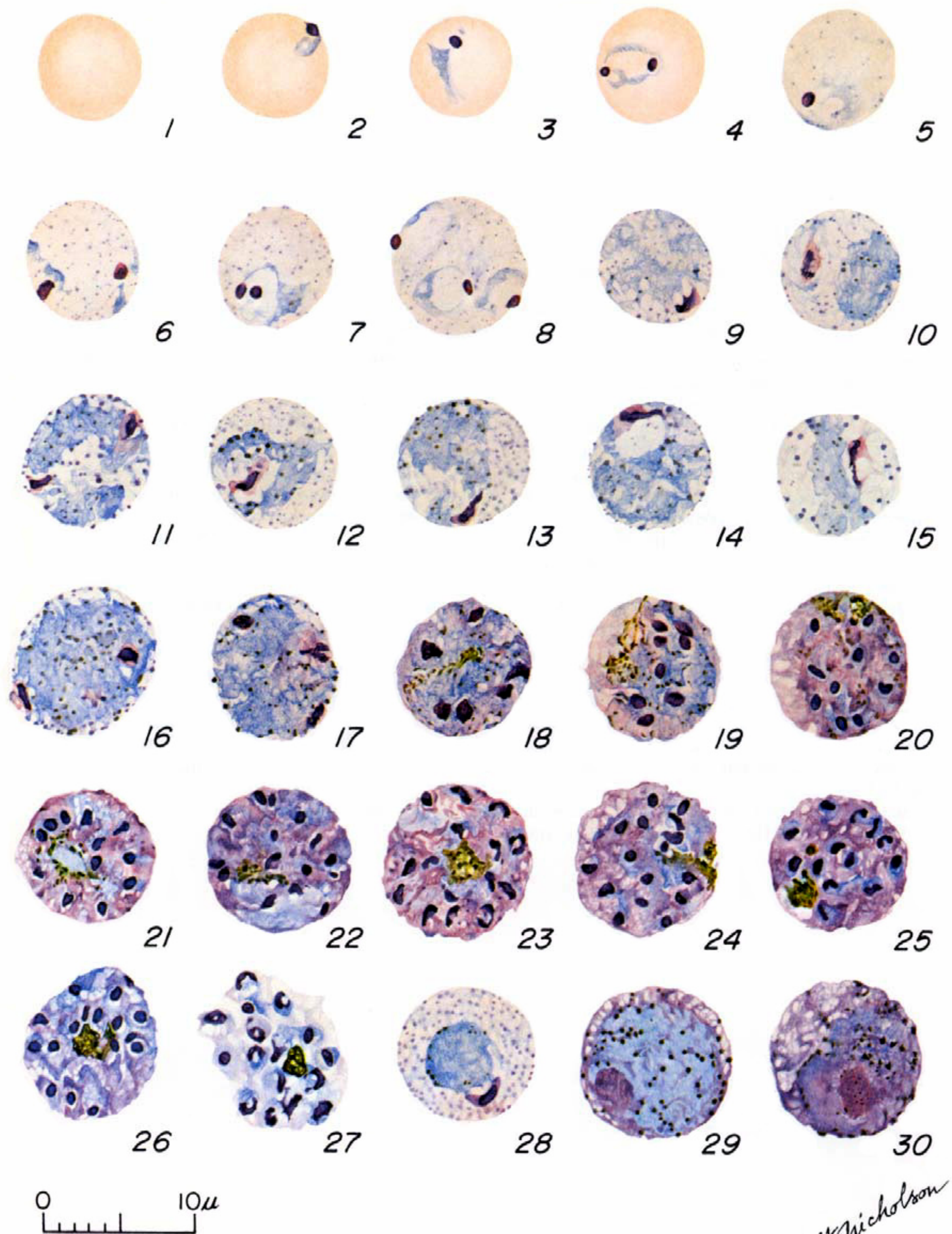
Plasmodium simium Fonseca, 1951

THERE is an interesting story connected with the discovery of *P. simium* as related by Dr. Deane (Deane *et al,* 1969) who, by his action, actually contributed to its discovery. In 1939 Fonseca was engaged in the study of the yellow fever virus in monkeys from the Itapecerica Forest near São Paulo, Brazil. An unusual temperature curve in the infected howler monkey *(Alouatta fusca)* prompted him to make smears of its blood. When he examined the preparations he came across a plasmodium which he assumed to be *P. brasilianum.* He made a series of smears over the next several days and saved them. Ten years later Deane, anxious to obtain a strain of *P. brasilianum,* asked Fonseca where the howler carrying the brasilianum parasite had been caught. This query prompted Fonseca to reexamine the original smears whereupon, to his surprise, he did not find *P. brasilianum,* as he had recorded in 1939, but a new parasite which he described and named *P. simium* in 1951. Garnham (1966) mentions that he and Fonseca studied the parasite again in 1955 in another young howler from the Itapecerica Forest.

In recent years Deane and his coworkers (1969) have made extensive studies of the malaria parasites in Brazilian monkeys, concentrating mainly on the southeast and north-west sections of the country. The studies show that *P. simium* occurs only in howlers *(A. fusca)* in the states of Rio Grande Do Sul, Santa Catarina, and São Paulo, but in the state of Esperito Santo it occurs not only in howlers but also in woolly spider monkeys *(Brachyteles arachnoides).* Adult howlers show infection more frequently than immature or very old ones; very young specimens are generally negative. Infections are present throughout the year but there is an increase in incidence during the summer. Why *P. simium* is limited to one small area of Brazil is an interesting question. The answer probably lies with a vector which occupies a very special ecological niche.



PLASMODIUM SIMIUM

Cycle in the Blood

PLATE XXII

The young parasites are rings, or modified forms, which may occupy about a fifth of the host cell, with a compact dark red nucleus and a rim of blue cytoplasm; these forms sometimes exhibit a double chromatin dot (Fig. 4). The developing parasites are amoeboid and retain the vacuole. Double infections are quite common (Fig. 8). Schüffner's stippling is prominent in all but the youngest rings. Some of these forms virtually fill the host erythrocyte (Figs. 9, 10). The young schizonts are marked by the disappearance of the vacuole; the nucleus elongates and divides; and small, barely visible pigment granules appear in the cytoplasm. In the older schizonts the nuclei take a deep reddish-purple stain, are large, oval or crescent-shaped. The mature schizonts display 12 to 18 merozoites. The schizonts are ragged in outline; medium to dark brown pigment is clumped into a mass, and sometimes these forms appear smaller than slightly younger forms (Fig. 21). The parasitized host cell increases in size about the time Schüffner's dots appear and later may reach a diameter of 11 μ.

The gametocytes have some resemblance to those of *P. vivax.* The young forms are compact with dense blue cytoplasm and a red-staining nucleus (Fig. 28). The mature gametocytes fill and distend the host cell. The macrogametocytes have a small dense-staining red nucleus; rather deep blue cytoplasm, and scattered, small granules of pigment (Fig. 29). The microgametocytes exhibit a diffuse pink nucleus, pale blue cytoplasm and small diffuse pigment granules (Fig. 30).

The asexual cycle in the blood is about 48 hours.

Sporogonic Cycle

PLATE XXIII

Although Deane *et al* (1966), on purely epidemiological grounds, forecast that *P. simium* was transmitted by *Anopheles cruzi,* the only studies reported on the sporogonic cycle have been with exogenous mosquito species. In our laboratory, (Collins *et al,* 1969 and later) we have allowed six different species of anophelines *(A. freeborni, A. maculatus, A. stephensi, A. quadrimaculatus, A. atroparvus,* and *A. b. balabacensis)* to feed on squirrel monkeys *(Saimiri sciureus)* infected with *P. simium.* Beginning as early as 3 days after infection, dissected midguts of the mosquitoes were examined to determine the presence and the diameters of the oocysts.

The results of the oocyst measurements are presented in Table 17. In *A. freeborni* at day 3, the mean oocyst diameter was 9 μ with a range of 7 to 11 μ. The oocysts continued to grow so that on day 12 they had an average size of 46 μ with a range of 22 to 63 μ. Sporozoites were first seen in the salivary glands on day 12.

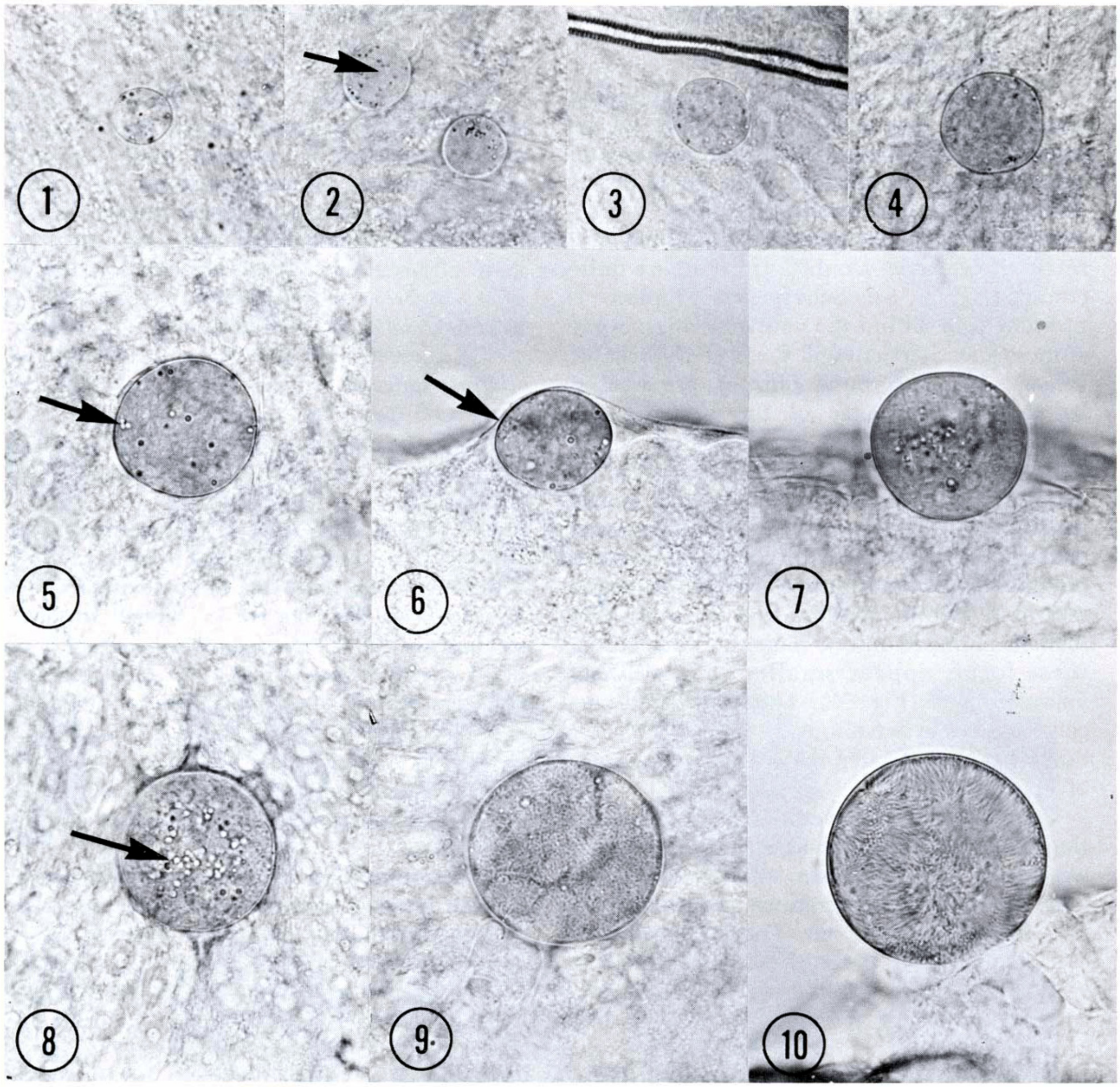
In *A. stephensi* the 5-day oocyst measured 13 μ with a range of 11 to 17 μ. On day 13 they averaged 53 μ, with a range of 24 to 71 μ. Sporozoites were present in the salivary glands on day 12.

The examination of the *A. maculatus* revealed a lower intensity of infection. On the 5th post-feeding day the oocysts had an average diameter of 12 μ with a range of 11 to 13 μ. On day 13, sporozoites appeared in the salivary glands and on that day oocysts had an average measurement of 47 μ with a range of 27 to 67 μ.

The number of oocysts available for measurement in *A. quadrimaculatus, A. atroparvus* and *A. b. balabacensis* was limited but the oocyst diameters in each of these species

PLATE XXII.--*Plasmodium simian*.

Fig. 1. Normal red cell.
Figs. 2-8. Young trophozoites.
Figs. 9-12. Growing trophozoites.
Figs. 13-15. Mature trophozoites.
Figs. 16-18. Young schizonts.
Figs. 19-21. Older schizonts.
Figs. 22-26. Nearly mature schizonts.
Figs. 27. Mature schizont.
Figs. 28-29. Young adult and mature macrogametocytes.
Fig. 30. Mature microgametocyte.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were within the range for the other test mosquitoes. Sporozoites were found in the salivary glands of *A. b. balabacensis* on day 12.

Plasmodium simium is a smaller parasite than *P. cynomolgi* and it takes about two days longer to complete its sporogonic cycle. This is shown graphically in Figure 34 where its growth curve in *A. freeborni,* the most acceptable test vector, is compared with that of *P. cynomolgi.* Heavy salivary gland infections were present in each of the test species by day 12.

The sporozoites in *A. freeborni* and in *A. maculatus* were fully viable and infective because bites by each of these species transferred sporozoites which initiated infection in splenectomized squirrel monkeys.

Parasites in 9 splenectomized squirrel monkeys were infective to *A. freeborni* mosquitoes when they were allowed to feed as early as 6 and as late as 41 days after onset of patent parasitemia. Oocyst densities of 5 or more per gut occurred in mosquitoes fed 6 to 21 days after onset of parasitemia. Indications are that the best infections result when feedings are carried out during the first 6 to 15 days of patent parasitemia.

Cycle in the Tissue

Fonseca reexamined tissue smears of the spleen, liver, brain, and kidneys of the original monkey whose parasites were seen in 1939 but was unable to find "elements of the exoerythrocytic cycle." We have sought these stages repeatedly, following heavy sporozoite inoculations in owl and squirrel monkeys. Only recently we have found 7-day forms in each of these animals; these forms are similar to *P. vivax* in the owl monkey.

Course of Infection

Until recently it was thought that the only natural host of *P. simium* was the black howler monkey *(Alouatta fusca)*; however, Deane *et al* (1968, 1968a) record finding this parasite as a natural infection in the woolly spider monkey *(Brachyteles arachnoides),* also. Deane and his coworkers (1969) were able to observe natural infections in several *A. fusca* for varying periods of time before splenectomy. All of the animals exhibited mild symptoms and moderate to light parasitemias. However, following splenectomy the parasitemia generally increased rapidly up to as high as 225,000 per mm^3 in about 3 weeks (Deane, 1964). He also reported anemia, loss of hair, diarrhea, and fever as high as 41.5° C. It must be kept in mind that howlers are difficult to keep in captivity and consequently some of these manifestations might have been due to their failure to accept food. In this connection Crandall (1964) reported that three howlers lived in captivity between 3 and 5 years. More recently, Malinow *et al* (1968) reported keeping two male howlers in good health, at the Oregon Regional Primate Center, for a period of two years. The latter investigators have since discontinued observations on this species because of the extreme mortality rate in captivity.

So far no one has reported the infection of the rhesus monkey with this parasite.

In the splenectomized squirrel monkey *(Saimiri sciureus)* Deane and Okumura (1965) were able to obtain heavy infections in three out of four animals infected by the inoculation of parasitized blood; in the fourth animal, the parasitemia was low and all evidence of infection had disappeared on the fourth day.

PLATE XXIII.—Developing ooccysts of *Plasmodium simium* in *Anopheles freeborni* mosquitoes. X 580 (Except Fig. 2).
Fig. 1. 4-day oocyst.
Fig. 2. 5-day oocysts showing scattered pigment. X 740.
Fig. 3. 6-day oocyst showing pigment and presence of small vacuoles.
Fig. 4. 7-day oocyst.
Fig. 5. 8-day oocyst showing prominent vacuoles.
Fig. 6. 9-day oocyst and mosquito gut membrane.
Fig. 7. 9-day oocyst.
Fig. 8. 10-day oocyst showing numerous small vacuoles.
Fig. 9. 10-day differentiating oocyst.
Fig. 10. 11-day fully differentiated oocyst.

TABLE 17.—Oocyst diameters of *Plasmodium simium* in *Anopheles freeborni, A. stephensi, A. b. balabacensis, A. quadrimaculatus, A. atroparvus,* and *A. maculatus.*

Days after Infection	*A. freeborni*			*A. stephensi*			*A. b. balabacensis*			*A. quadrimaculatus*			*A. atroparvus*			*A. maculatus*		
	No.	Range*	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
3	8	7-11	9															
4	97	9-20	14															
5	200	8-22	14	100	11-17	13	2	14-17	15	6	13-19	17				16	11-13	12
6	201	12-32	19	111	12-26	17	27	13-27	19	25	13-24	18				17	11-18	15
7	136	13-35	23	126	18-39	25				3	24-26	25	12	20-38	28	20	12-35	20
8	132	13-49	26	111	14-53	27	18	20-44	34	28	26-47	35	85	25-53	39	48	15-51	28
9	120	17-54	31	103	19-44	33	8	26-46	36	58	26-59	41	30	20-55	39†	92	14-53	32
10	196	18-55	42†	201	20-61	47†	33	26-48	38	33	31-59	46†	32	24-57	39†	105	18-60	41†
11	110	21-60	47†	200	30-63	49†	20	31-54	43†	34	32-73	47†	13	26-63	49†	66	27-63	49†
12	100	22-63	46†**	194	18-64	46†**	2	40-59	50†**	6	51-63	57†				86	24-71	48†
13				106	24-71	53†**				5	30-65	47†				48	27-67	47†**
Totals	1300	7-63		1252	11-71		110	13-59		198	13-65		172	20-63		498	11-71	

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

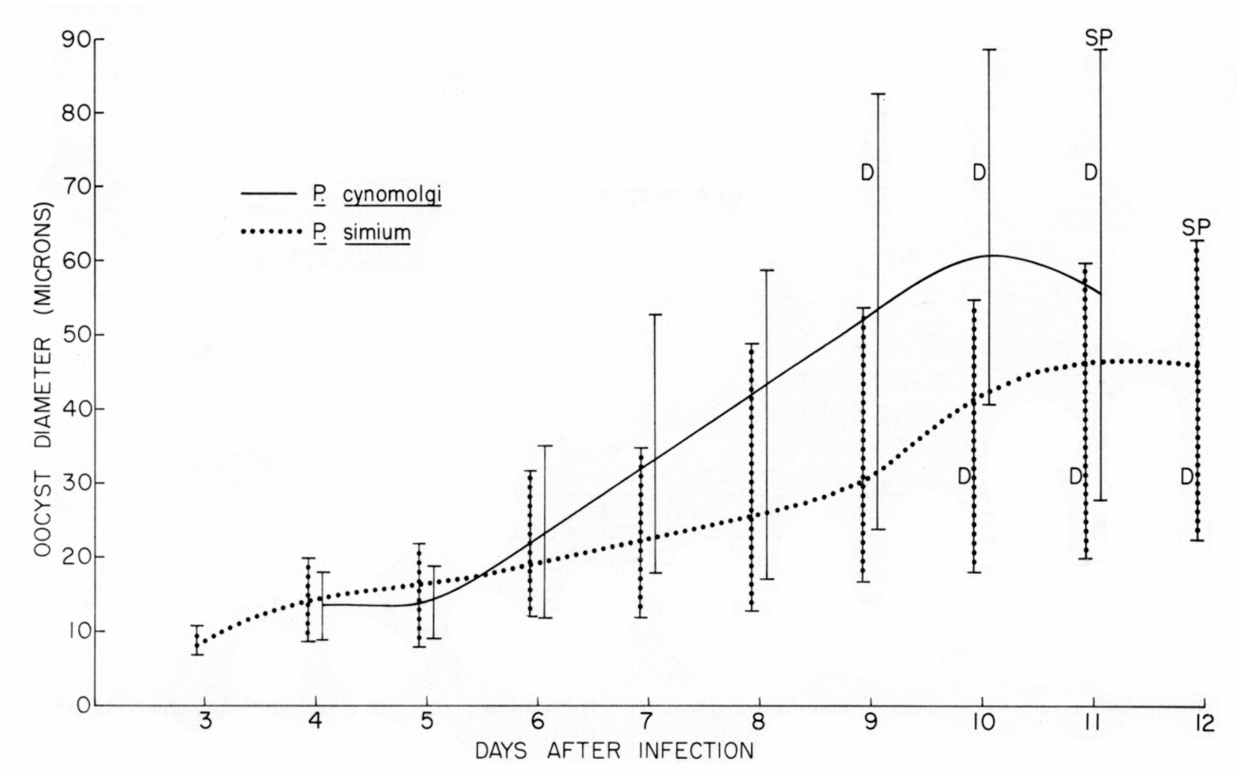

FIGURE 34.—Mean growth curves and ranges in oocyst diameters of *Plasmodium simium* and *P. cynomolgi* in *Anopheles freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

Less severe infections were obtained in *A. paniscus* and in young *A. fusca* (Deane *et al,* 1966a) and mild infections in *Lagothrix lagotricha* (Deane *et al,* 1965a). The marmoset, *Catticebus iacchus,* was found susceptible but the infection was of low order (Deane and Okumura, 1965b). All the animals, as reported by the Brazilian workers, recovered from their infections spontaneously except a single very young specimen of *S. sciureus.*

In our own studies, infection was obtained in 12 splenectomized squirrel monkeys by the inoculation of parasitized blood (Fig. 35). The parasitemia rose rapidly to a median count greater than 10,000 per mm^3 by day 8 and remained at that level or higher for approximately 25 days. The highest parasitemia, approximately 440,000 per mm^3 was obtained in one animal 22 days after inoculation. In another, the parasite count dropped to zero on day 26; 9 of the 12 test animals were negative by day 60. Patent infections persisted in the other three for as long as 112 days.

We were able to transmit the parasite to two splenectomized S. *sciureus* through the bites of *A. maculatus* in one and *A. freeborni* in the other; the prepatent periods were 24 and 38 days, respectively. The maximum parasite counts in these animals ranged from 63,000 to 160,000 per mm^3 of blood. The animal with the highest parasite count died; in the other, parasitemia persisted for 100 days.

In 1966b Deane *et al* reported that one of the members of his research crew, working in the government forest reserve outside São Paulo, had developed fever with a tertian pattern (up to 39.5° C) which lasted about a week. Smears of the man's blood showed scanty parasites of malaria. Some of his blood was given to a splenectomized squirrel monkey which developed a high parasitemia. The authors attributed the human infection to *P. simium* because the parasite was *P. vivax-like,* and, because it grew well in *S. sciureus.* In our opinion it could just as well have been *P. vivax* because in the same year (1966b) Deane *et al*

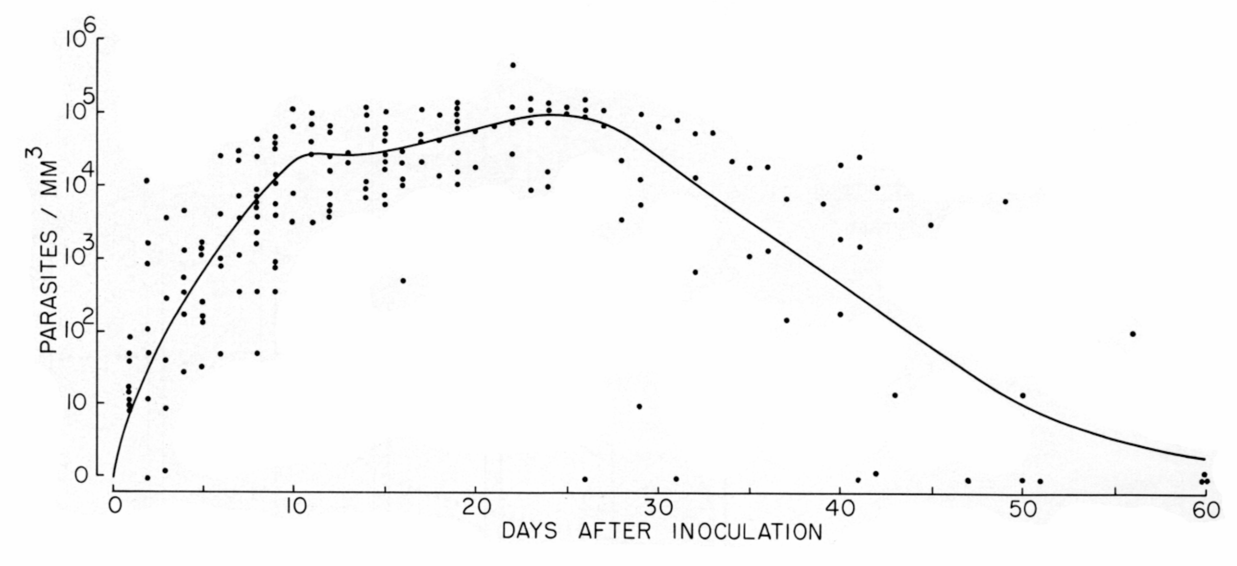

FIGURE 35.—Parasite counts with the median curve of parasitemia for *Plasmodium simium* in 12 splenectomized squirrel monkeys, *Saimiri sciureus*, inoculated with parasitized blood.

showed that *P. vivax* would grow in splenectomized squirrel monkeys. Furthermore, in the following year, using a strain of *P. simium* received from Dr. Deane, we failed in five attempts to infect human volunteers, via mosquito bite, employing *A. stephensi* and *A. freeborni.* In three of these attempts, infectivity of the sporozoites was confirmed by their ability to initiate infection in splenectomized squirrel monkeys. Since these trials, we have learned that by the use of splenectomized Saimiri (squirrel) monkeys sporozoites of *P. simium,* produced by these mosquitoes, are routinely infective. In view of these considerations the acceptance of a *P. simium* infection occurring naturally in man should be held in abeyance until there is more evidence in support of its zoonotic nature.

Host Specificity

The natural vector *of P. simium* is unknown. In 1966a, Deane *et al* incriminated *A. cruzi* as the probable natural vector because it occurs in the area where there are infected howler monkeys and because it feeds in the forest canopy as well as at ground level. In a more recent paper summarizing all his studies through July, 1968 (Deane *et al,* 1969) he contends "one can hardly incriminate another mosquito. ..". It may well be that *A. cruzi* is the vector and the proof of its ability may come shortly. However, when sporozoites from *A. cruzi* were transferred to three different squirrel monkeys, patent infection did not occur. As mentioned earlier, we were able to infect six species of anophelines with this parasite. The intensity of infections in the mosquitoes varied from one species to another (Table 18). *Anopheles freeborni* was the most acceptable host followed by *A. stephensi, A. maculatus, A. b. balabacensis, A. quadrimaculatus,* and finally *A. atroparvus.* Average oocyst densities greater than one per gut in *A. freeborni* may be expected to yield salivary gland infections of 50 percent or greater after an incubation period of 14 days and a temperature of 25° C.

Antigenic Relationships and Immunity

Two splenectomized *S. sciureus* monkeys were inoculated with sporozoites of *P. simium* but failed to develop a patent infection with that species. One of the animals was splenectomized two days prior to inoculation and the other 18 days after inoculation. Subsequent to the splenectomy, each developed a patent infection with *P. brasilianum* rather than the expected *P.*

TABLE 18.—Comparative infectivity of *Plasmodium simium* to *Anopheles freeborni, A. stephensi, A. b. balabacensis, A. quadrimaculatus, A. atroparvus,* and *A. maculatus*.

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
F-1						100
F-1 : St-1	20	269	332	38.3	25.3	46.2
F-1 : Bal	9	81	173	60.5	20.2	10.3
F-1 : Q-1	12	89	248	78.7	20.2	10.1
F-1 : Atro	6	43	151	83.7	19.9	8.6
F-1 : Mac	26	286	670	51.7	19.7	8.1

* F-1 = *Anopheles freeborni,* St-1 = *A. stephensi,* Bal = *A. b. balabacensis,* Q-1 = *A. quadrimaculatus,* Atro = *A. atroparvus,* Mac = *A. maculatus.*

** 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.

simium. It may well be that a latent *P. brasilianum* will prevent the development of a patent *P. simium* infection. Also, in this connection, we have failed to obtain patent *P. simium* infections in intact squirrel monkeys by the inoculation of sporozoites indicating either the necessity for splenectomy or the presence of a latent infection with *P. brasilianum.*

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