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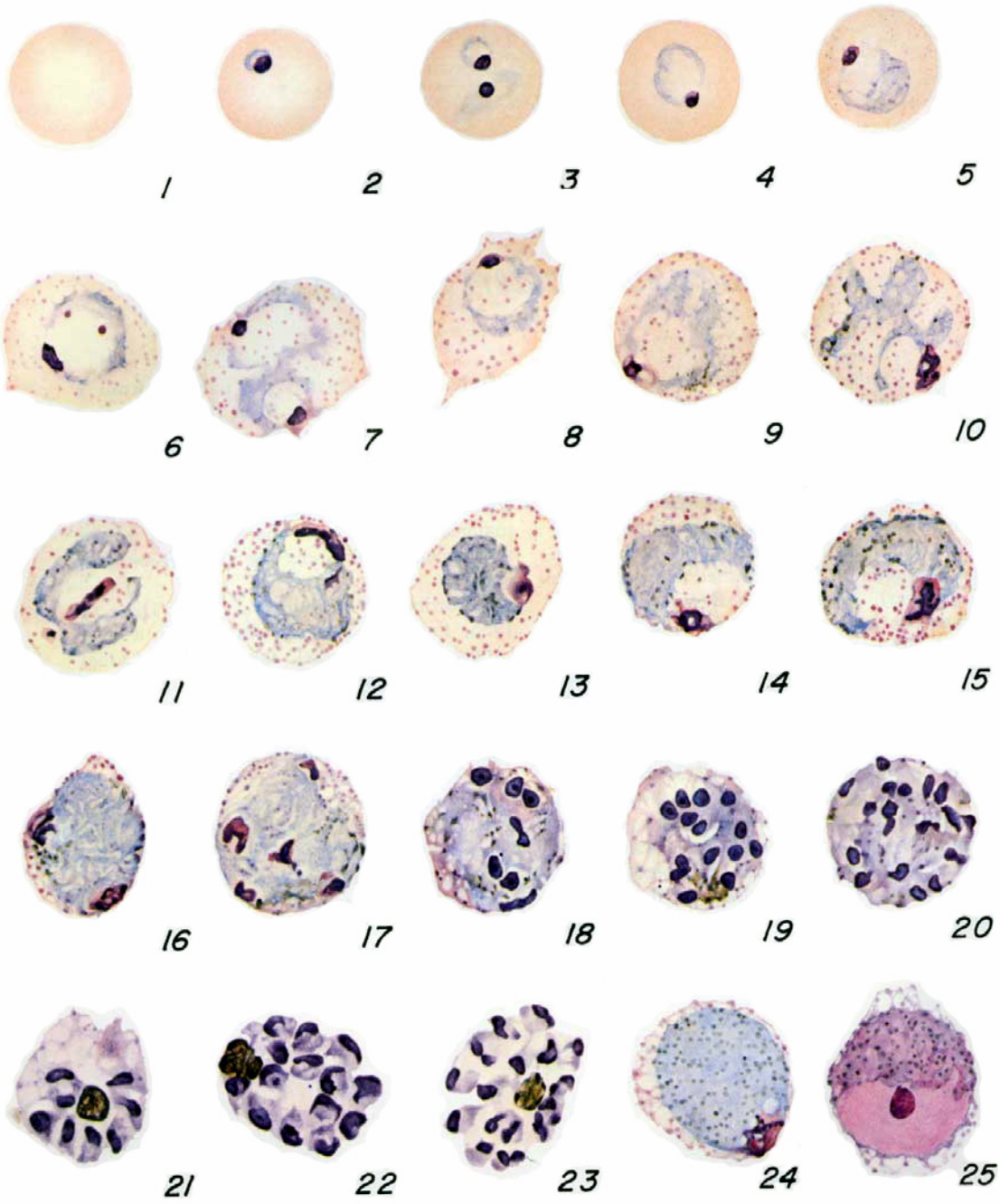
Plasmodium gonderi Sinton and Mulligan, 1933

THIS parasite was first seen by Gonder and von Berenberg-Gossler (1908) in the blood of a mangabey, *Cercocebus fuliginosus* (= *C. atys)*, housed in the Hamburg Zoo. These authors identified the organism as *Plasmodium kochi.* Berenberg-Gossler saw the parasite again in 1909 in the same host, and, in two long-tailed green monkeys which Sinton and Mulligan (1933) identified as *Cercopithecus (C. sabaeus).* Each of the Berenberg-Gossler papers concerned with this parasite was illustrated with beautiful colored plates comprising some 72 figures. In 1910, Gonder and Rodenwalt again studied the parasite in the natural host and pointed out its morphological resemblance to *P. vivax* and its tertian periodicity.

Sinton and Mulligan in 1932-33 did a complete review of all the known malarias from the lower monkeys (*Cercopithicidae* and *Colobidae)* and concluded that the parasite described by Gonder and von Berenberg-Gossler was a true *Plasmodium* allied to the *P. inui* group. They proposed the name *P. inui gonderi.* At that time, it was not recognized that *P. inui* had a 72-hour cycle. Rodhain and van den Berghe (1936) isolated the parasite from a *Cercocebus galeritus agilus* from the Congo and proceeded to study it. They, as Gonder and Rodenwalt before them, found its periodicity to be tertian. This being true, it could not be considered as belonging to the inui group of malaria parasites and, therefore, they raised it to specific rank under the name *Plasmodium gonderi.*

According to Garnham *et al* (1958), Duke, in 1956, found a malaria parasite in a drill *(Mandrillus leucophaeus),* taken in the Cameroons, and succeeded in infecting other drills with it. An infected animal was eventually sent to Garnham who, after careful study, concluded that the parasite was *P. gonderi.* The significance of this find was that it expanded the range of the parasite some one thousand miles northwest of its then known habitat. The following year (1959) Bray encountered the same parasite in mangabeys *(Cercocebus fuliginosus)* in Liberia which further extended its range to include the west coast of Africa from the mouth of the Congo river to Liberia.



PLASMODIUM GONDERI

G. H. Nicholson

Cycle in the Blood
PLATE IX

The young merozoites invade the blood stream and prefer to enter reticulocytes, according to Rodhain and Lassman (1939) where they are seen as small bodies which quickly grow into the "signet ring" stage. These young forms, sometimes two in a cell (Fig. 3), or as many as four, show a pale blue cytoplasm and a deep red nucleus with no enlargement of the host cell (Figs. 2-4). With further growth of the parasite, Schüffner's stippling appears in the cytoplasm. The host cell is increased in size and there may be some distortion (Figs. 5, 6, 8). The parasite displays a large vacuole, except in the amoeboid forms (Fig. 10) and fine to granular greenish-brown scattered pigment which may be located along the periphery. In the older forms, the cytoplasm stains a deeper blue; the nucleus is larger, irregular to bar-shaped, and takes a deep red stain, often with a lighter stained area. The pigment is now in small aggregates (Figs. 14, 15) which Rudzinska *et al* (1960) showed by electron microscopy were actually in individual food vacuoles due to intracellular phagotrophy. Host cell stippling is prominent (Figs. 11-14).

Further growth produces the young schizont. In the early nuclear divisions, the chromatin appears as flat-oval to irregular red masses located on the periphery of the parasite (Figs. 16, 17). The pigment is more condensed but scattered. Stippling is prominent. The older schizont may almost fill the host cell and shows a purplish cytoplasm with deeper reddish-purple nuclei. The nuclei still appear as if on the periphery of the parasite and some look as if they were about to escape from it (Fig. 20). The mature schizont may not fill the host cell. There may be 12 to 20 merozoites, generally about 16, and each one appears to have a purple-staining broad end which occupies 1/3 to 1/2 of the body with a lighter trailing area sometimes with only a suggestion of a vacuole. The pigment is clumped into a yellow-gold to black central mass. The cytoplasm of the host cell is hypochromic almost to the point of being inapparent so that the schizont may appear free (Figs. 22, 23).

The young gametocytes appear as heavy rings with large deep-staining nuclei. As the parasite grows, the cytoplasm becomes dense and the presence of prominent dark pigment sets it off from the asexual parasites. The macrogametocyte stains a deep blue with scattered pigment. The nucleus, generally eccentric, stains a deep red generally with a lighter portion toward the center. The parasite may not entirely fill the enlarged host cell (Fig. 24). The mature microgametocyte stains a light purplish-pink with dark pigment granules scattered in the cytoplasm with some tendency to collect toward the periphery. The nucleus is somewhat diffuse and occupies a large part of the parasite; it takes a slightly purplish-pink stain and encloses a deep red oval body. This parasite, too, may fill the host cell which is somewhat enlarged (Fig. 25).

The parasite has a 48-hour cycle.

Sporogonic Cycle
PLATE X

According to Garnham (1966), each gamete produced by the exflagellating gametocyte measures about 16 μ in length with a dot at one end, and, what is taken to be, two nuclei near the center. The ookinetes appear in the gut of the mosquito at about 21 hours after feeding. The parasite is about 12 μ long and, when fully mature, pigment collects at the broad end as a brown mass in a yellowish sac. A clear circular

PLATE IX.—*Plasmodium gonderi.*

Fig. 1. Normal red cell.
Figs. 2-4. Young trophozoites.
Figs. 5-11. Growing trophozoites.
Figs. 12-15. Mature trophozoites.
Figs. 16-20. Developing schizonts.
Figs. 21-23. Mature schizonts.
Fig. 24. Mature macrogametocyte.
Fig. 25. Mature microgametocyte.

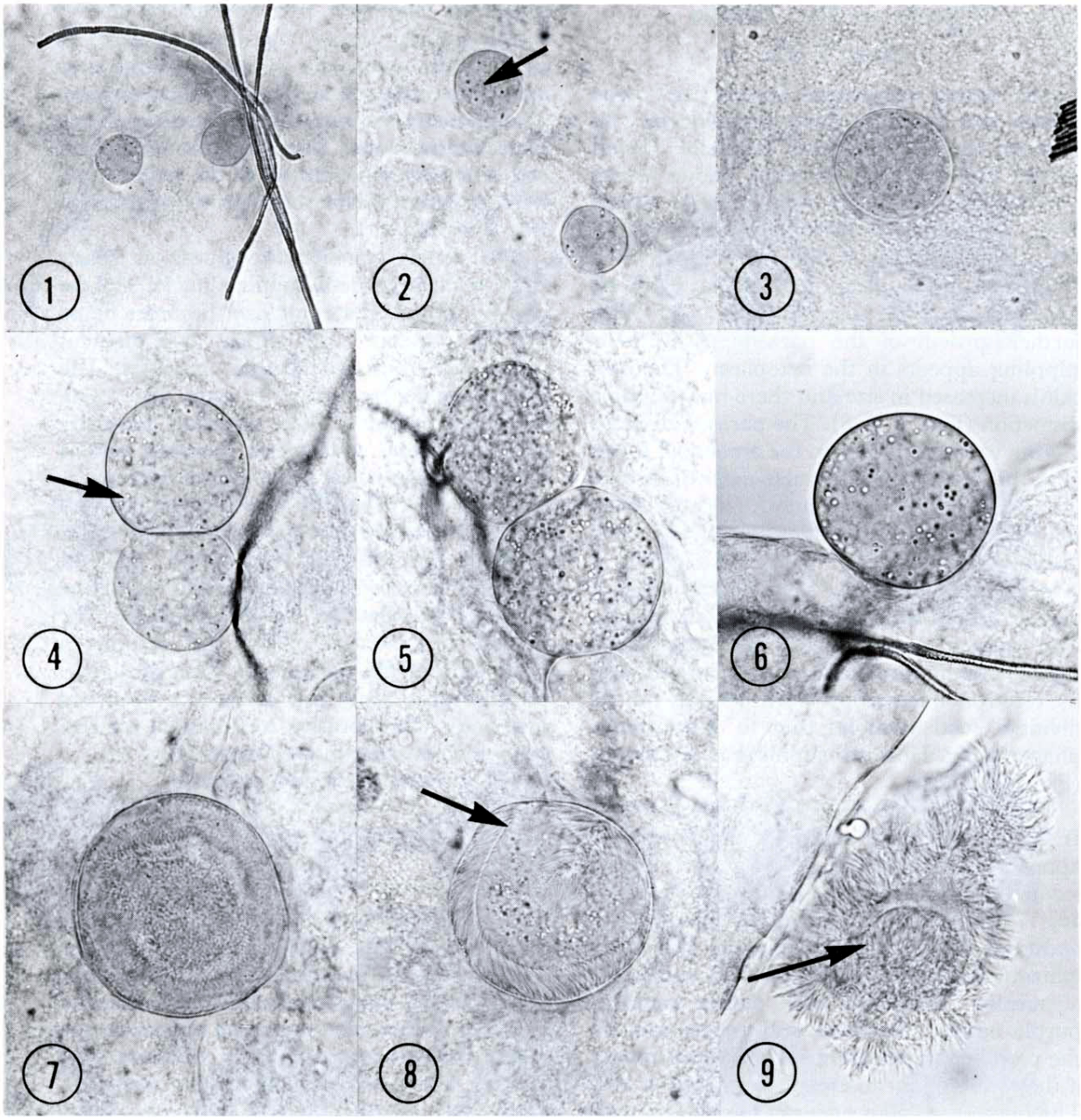


PLATE X.—Developing oocysts of *Plasmodium gonderi* in *Anopheles freeborni* mosquitoes. X 580.

Fig. 1. 5-day oocyst.
Fig. 2. 6-day oocyst showing scattered pigment.
Fig. 3 7-day oocyst.
Fig. 4. 8-day oocyst showing small vacuoles.
Fig. 5. 9-day oocyst showing a large number of small vacuoles.
Fig. 6 10-day oocyst.
Fig. 7 10-day differentiating oocyst.
Fig. 8. 11-day oocyst showing atypical form of differentiation.
Fig. 9. Prematurely ruptured 11-day oocyst showing sporozoites attached to sporoblastoid body.

vacuole is generally present in the cytoplasm; the nucleus is not too well defined.

Bano (1959) studied the early sporogony of *P. gonderi* in *Anopheles aztecus* and reported that at 50 hours the oocysts measured about 12 µ in diameter. At 58 hours, as a result of mitosis, the haploid number of chromosomes was shown to be three (two large and one small). Growth proceeds rapidly at 28° C. At 6 days, the oocysts measured 25 µ, at 7 days 40 µ and when mature, up to 60 µ. Nine days after the blood meal, sporozoites, measuring about 10 µ in dried films, were in the salivary glands.

We have observed the oocyst growth in six species of anophelines when incubated at 25° C (Table 10). In *A. freeborni,* the mean oocyst diameter at day 4 was 13 µ with a range of 9 to 19 µ. The oocysts continued to grow so that by day 12, the mean diameter was 65 µ, with a range of 41 to 94 µ, and sporozoites were present in the salivary glands.

In the other mosquitoes, *A. stephensi, A. maculatus, A. b. balabacensis, A. quadrimaculatus,* and *A. atroparvus,* the oocyst diameters were within the limits found in *A. freeborni.* Sporozoites were present in the salivary glands of the *A. quadrimaculatus* and *A. atroparvus* on day 12; in *A. maculatus* on day 13; and in *A. stephensi* on day 15. Although the oocyst development appeared to be normal in the *A. b. balabacensis,* sporozoites did not appear in the salivary glands until day 16, and in some lots, not until day 20.

A comparison of the oocyst growth curve of *P. gonderi* with that of *P. cynomolgi* (Fig. 27) indicates that these species in *A. freeborni* are similar in size through 11 days of incubation. However, sporozoites were present in the salivary glands of mosquitoes infected with *P. cynomolgi* two days earlier than with *P. gonderi.*

The sporozoites were shown to be infective because the infection was transmitted to rhesus monkeys by the bites of *A. freeborni* (3 times), *A. maculatus* (once), *A. stephensi* (3 times), and *A. b. balabacensis* (twice). The prepatent periods of these nine transmissions ranged from 9 to 17 days with a mean of 12.7 days. Garnham *et al* (1958) reported prepatent periods of 8 days in each of two *M. mulatta* monkeys infected by the intravenous inoculation of infected salivary glands from *A. aztecus* mosquitoes.

Cycle in the Tissue

We have tried to demonstrate the EE cycle of *P. gonderi* on one occasion but met with

TABLE 10.--Oocyst diameters of *Plasmodium gonderi* in *Anopheles freeborni, A. stephensi, A. maculatus, A. b. balabacensis, A. quadrimaculatus,* and *A. atroparvus.*

Days after Infection	*A. freeborni*			*A. stephensi*			*A. maculatus*			*A. b. balabacensis*			*A. quadrimaculatus*			*A. atroparvus*		
	No.	Range*	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
4	100	8-19	13	100	9-25	14	100	8-18	13	100	8-19	13	100	9-26	16			
5	100	11-25	19	100	12-31	22	100	12-27	20	100	12-26	20	100	12-26	19	8	14-18	16
6	204	12-34	23	100	18-32	25	222	12-32	23	216	12-33	23	102	18-33	25	49	18-31	24
7	100	13-30	22				88	15-27	21	111	18-35	28	100	14-28	22			
8	100	18-51	37				100	21-54	38	121	18-59	39	101	18-50	32	99	20-55	40
9	100	30-64	50	41	22-41	33	100	24-61	44	100	24-57	42	101	24-53	39	158	24-63	44
10	100	24-72	58	100	30-77	53	100	24-72	45	100	24-77	50	100	24-72	50	8	40-59	48
11	100	30-85	56†	100	41-89	62†	100	30-65	50	100	41-85	61†	100	35-83	60†	100	33-70	49†
12	100	41-94	65†**	100	30-77	55†	100	35-89	61†	100	41-94	63†	100	35-85	63†**	100	30-80	57†**
13	100	41-89	68†**	100	22-89	59†	100	41-107	70†**	90	35-104	71†	100	18-100	61†**			
14	100	35-100	71†**	100	30-94	65†	100	33-94	63†**	100	34-94	66†	100	51-98	71†**			
15	100	44-81	67†**			**												
Totals	1304	8-100		841	9-94		1210	8-107		1238	8-104		1104	9-100		431	14-80	

* Measurements expressed in microns.
† Oocyst differentiation.
** Sporozoites present in the salivary glands.

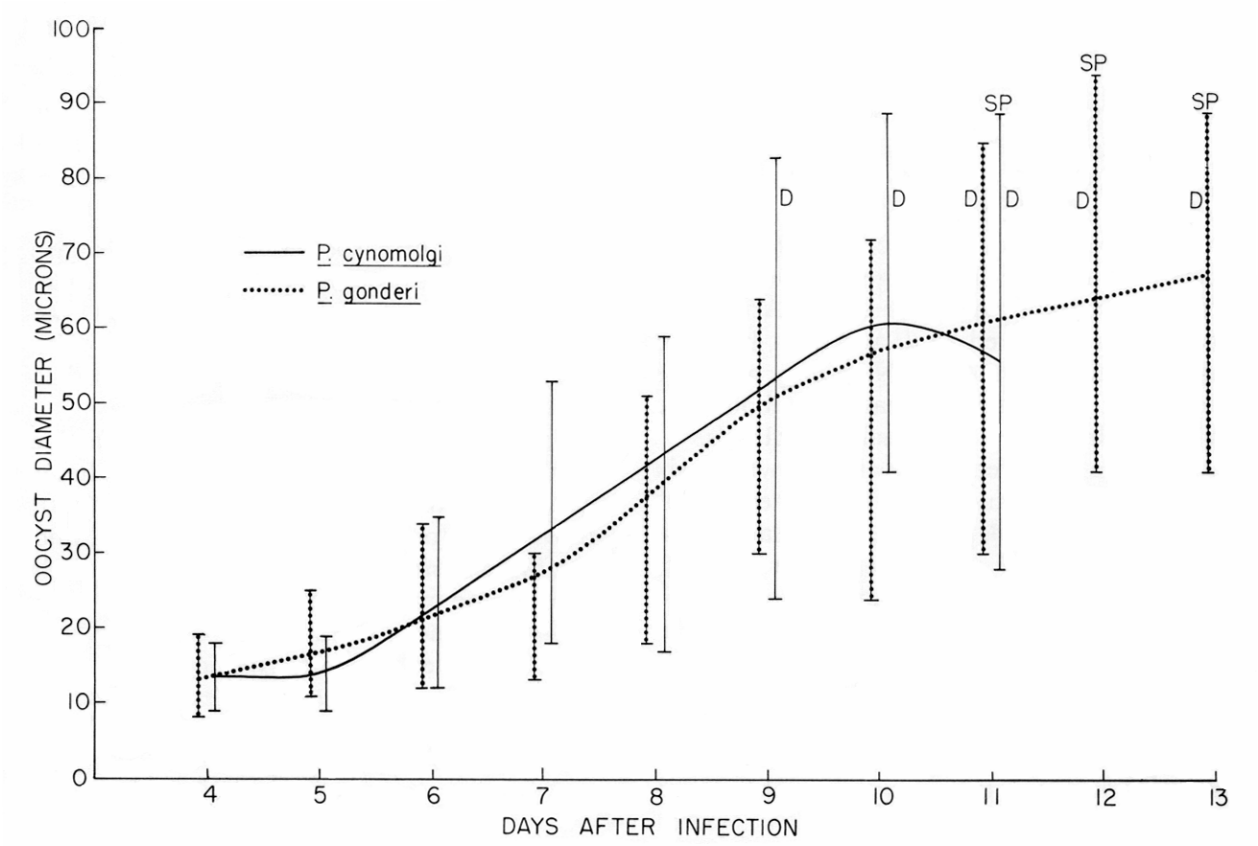


FIGURE 27.—Mean oocyst growth curve and ranges in oocyst diameters of *Plasmodium cynomolgi* and *P. gonderi* in *Anopheles freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

failure even though the test animal developed an infection after a suitable prepatent period. Garnham *et al* (1958) were able to demonstrate the EE cycle in the rhesus monkey on days 5, 7, and 8 and reported that the 5-day schizonts were within a definite limiting membrane surrounding a granular cytoplasm with about 20 nuclei. The largest schizont measured 13 by 19 μ. Seven-day forms were generally oval in shape with a regular outline. The largest ones measured 18 to 27 μ. These forms showed numerous large dense spherical to rod-shaped nuclei; the cytoplasm was granular and without vacuoles.

The eight-day forms represented nearly mature, mature, and post-mature parasites. They expressed no decided increase in size over the 7-day forms but there were many more nuclei packed closely together. The nuclei were small and appeared as densely stained granules in a paler matrix. Some of the schizonts showed lobations. The cytoplasm was granular and contained in an oval outline. The size of these bodies was variable, the largest was 27 by 32 μ, and estimated to carry about two thousand merozoites.

It is not known if there are secondary EE bodies in the life-cycle of *P. gonderi.*

Course of Infection

According to Garnham *et al* (1958), blood-induced infections in the rhesus monkey are characterized by initial high parasitemias which decline slowly during the following weeks and then persist; parasites were found easily, in thin films, after twelve months. The peak of schizogony was found to occur about mid-day. Infected animals showed no signs of illness. Zuckerman (1960), on the other hand, reported that *P. gonderi* produced an excessive degree of anemia in these animals.

In our studies, the course of infection was followed in 25 *M. mulatta* monkeys; 17 were infected by the inoculation of parasitized blood and 8 by the inoculation of sporozoites (Fig. 28). In the former animals, the peak parasitemia (approximately 140,000/mm^3) occurred after about 10 days of parent parasitemia. The parasite count then declined slowly to a more or less persistent level. After 60 days of patent parasitemia, the median count was about 5,000/mm^3.

In the 8 monkeys infected by sporozoite-inoculation, the peak parasite count obtained on the 10th day of patent parasitemia (approximately 190,000/mm^3). The parasite count then declined but to a lower level than found in the blood-induced infections. The median parasite count at 60 days was about 350/mm^3. None of the animals required chemotherapy for survival.

Host Specificity

The natural hosts of *P. gonderi* are the mangabeys and drills found on the west coast of Africa and in the Cameroons. It was reported from mangabeys, *Cercocebus fuliginosus* (= *atys)* by Gonder and von Berenberg-Gossler (1908), and from C. *galeritus,* C. *aterrimus,* and C. *atys* by Bray (1963). Garnham *et al* (1958) reported it from the drill, *Mandrillus leucophaeus.*

Numerous investigators have successfully transferred *P. gonderi* to the rhesus monkey, *M. mulatta,* via blood and by sporozoites. It was passaged by blood to *Papio anubis, P. jubilaeus,* and *Cercopithecus aethiops* by Rodhain and van den Berghe (1936). We have also infected *M. radiata* by blood inoculation.

Rodhain and van den Berghe (1936) made one attempt to transfer *P. gonderi* to man by blood inoculation but without success. We attempted to transmit this species to 8 volunteers by mosquito bite; all attempts failed with observation continued for 180 days.

The natural vector of *P. gonderi* is unknown, but being an African-based parasite, it would be expected that *A. gambiae* would transmit the infection readily. However, Bray (1959), given favorable conditions was unable to obtain infections. Rodhain and van Hoof (1940) successfully transmitted the infection using *A. atroparvus.* Garnham *et al* (1958) obtained transmission using *A. aztecus.* We have been able to infect *A. freeborni, A. maculatus, A. b. balabacensis, A. stephensi, A. quadrimaculatus, A. atroparvus, A. sundaicus,* and *A. albimanus.* The level of susceptibility varied (Table 11), with *A. freeborni, A. b. balabacensis, A. maculatus, A. stephensi,* and *A. quadrimaculatus* being readily susceptible whereas the other species were not.

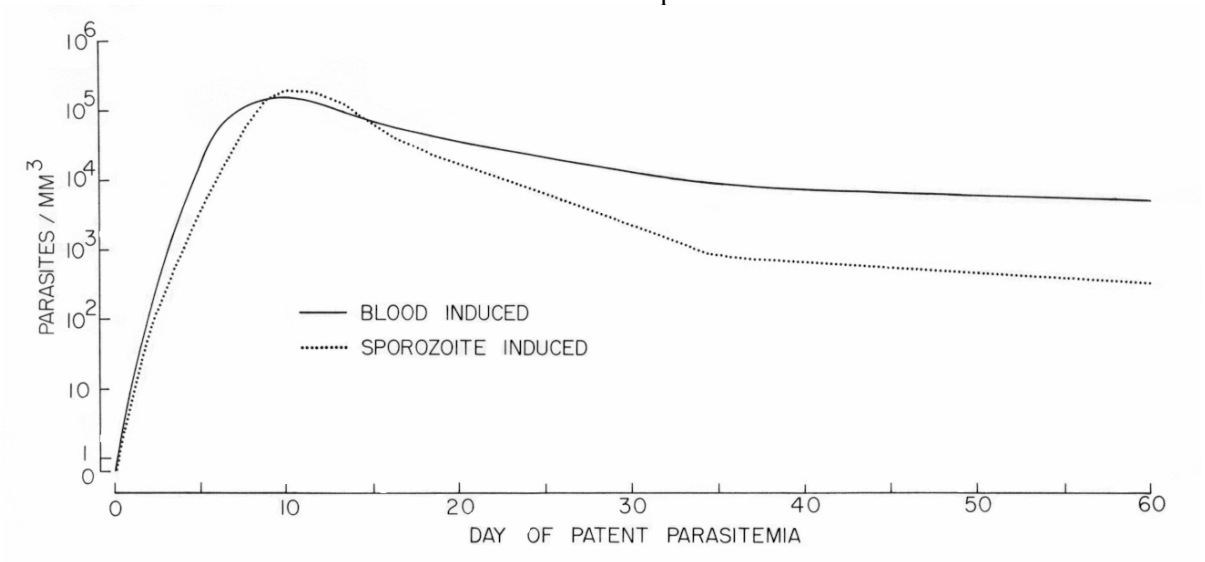


FIGURE 28.—Median curves of the parasitemia of blood-induced (17 animals) and sporozoite-induced (8 animals) infections of *Plasmodium gonderi* in *Macaca mulatta* monkeys.

Immunity and Antigenic Relationships

Garnham and Bray (1955) reported no cross-immunity between *P. gonderi* and *P. cynomolgi* in that a monkey cured of one infection was completely susceptible to infection with the other species. Voller *et al* (1966) reported that monkeys originally infected with *P. cynomolgi* or with *P. knowlesi* could be infected with *P. gonderi* and that a normal infection developed.

Data from serology show that antisera to *P. gonderi* give a fluorescent antibody cross-reaction at only a low level to *P. fieldi* antigen (mean reciprocal titer ratio of 100:41) and much lower levels of reactivity to other primate malaria antigens (Collins *et al,* 1966). In the reverse procedure, *P. gonderi* antigen produces low level responses to *P. cynomolgi, P. fragile,* and *P. inui* antisera (mean reciprocal titer ratios of 100:36, 100:35, and 100:32, respectively).

Although antisera to *P. falciparum* and *P. malariae* responded to the *P. gonderi* antigen, the cross reactions were much lower than the homologous responses (Collins *et al,* 1966a).

TABLE 11.—Comparative infectivity of *Plasmodium gonderi* to eight species of *Anopheles.*

Mosq. species Comparison*	Number tests	Number mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
F-1						100
F-1 : Bal	11	121	106	50.4	44.3	87.0
F-1 : Mac	22	245	236	58.8	64.4	84.7
F-1 : St-1	9	69	82	73.9	75.6	55.4
F-1 : Q-1	28	563	569	59.0	43.8	50.6
F-1 : Atro	7	210	186	58.6	8.6	6.5
F-1 : Sund	7	178	143	64.0	9.8	2.6
F-1 : Alb	9	124	117	25.8	2.6	0.3

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