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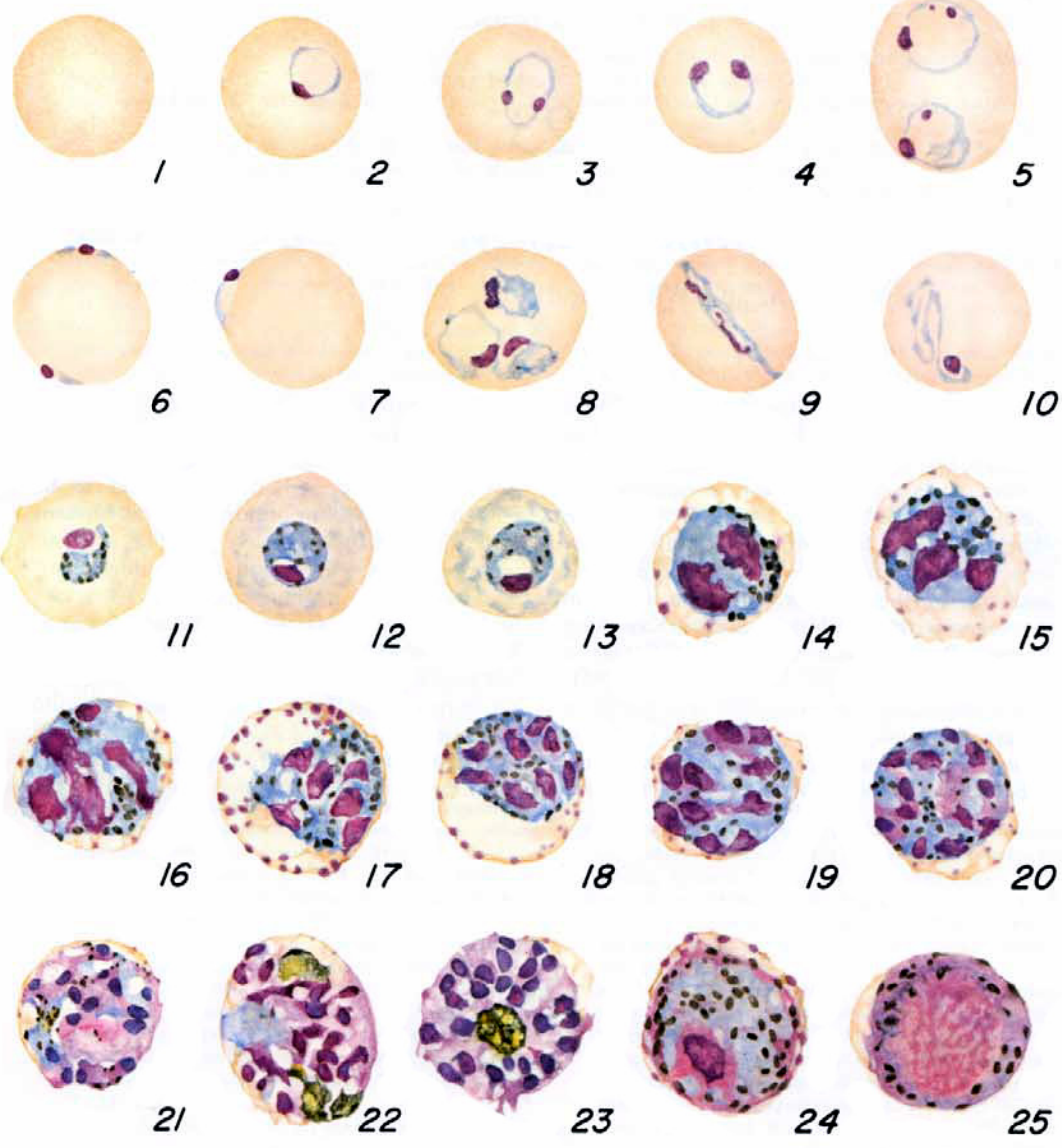
Plasmodium coatneyi Eyles, Fong, Warren, Guinn, Sandosham, and Wharton, 1962

IN the course of studies on simian malaria begun by the late Dr. Don Eyles in Malaya, he and his co-workers isolated a new species of malaria from a wild caught anopheline mosquito, *Anopheles hackeri.* This unique experience is the first instance of finding a new species of malaria in the vector before it was known from the primate host. The mosquito was taken in the nipah palm area of Kampong Rantau Panjang near the town of Klang in the State of Selangor in November, 1961. Upon dissection, the mosquito was found to be positive with sporozoites which were injected into an uninfected rhesus monkey, *Macaca mulatta,* without delay. The monkey exhibited infection after a prepatent period of 14 days. The parasite was first taken to be *Plasmodium knowlesi* on morphological grounds but when the periodicity was found to be tertian, rather than quotidian, it was obvious that the investigators were dealing with an undescribed species which they named *Plasmodium coatneyi* in honor of the American malariologist Dr. G. Robert Coatney. Later, Eyles *et al* (1962) isolated *P. coatneyi* from a kra monkey, *M. irus* (= *fascicularis)* taken in the same area as the original infected mosquito and then again in 1963 (Eyles *et al,* 1963) from the blood of an *M. irus* (= *fascicularis)* from the Philippines.



PLASMODIUM COATNEYI

G. H. Nicholson

Cycle in the Blood

PLATE XLV

The young ring forms of *P. coatneyi* closely resemble those of the human parasite, *P. falciparum.* The youngest ring forms are smaller than the rings of other simian parasites (not shown on plate) except, possibly, *P. knowlesi.* The typical young trophozoite has a single or double chromatin body (Figs. 2-5) but they may number up to four. The position in the host cell is varied as is true with the same age growth forms of *P. falciparum.* Marginal, appliqué or accolé forms are common (Fig. 6), often considered diagnostic of *P. falciparum* along with displaced vesicular forms (Fig. 7) with one or two nuclei. In heavy infections, host cells may harbor two or more young parasites (Fig. 8); band and tenue forms are not uncommon (Figs. 9, 10). In some instances the host cell carrying the young parasites is smaller than the normal cell.

As the trophozoites mature, their number in the peripheral blood becomes less. The usual form is circular or oval, usually with a vacuole and with intense blue cytoplasm (Figs. 11-13). The youngest of these forms rarely show pigment, but as the parasite grows the granules become prominent; they do not coalesce. Maurer's spots or clefts are prominent and characteristic of this species. These were originally described by Eyles *et al* (loc. cit.) and their fine structure more recently by Rudzinska and Trager (1968). The bluish cast to the cytoplasm in Figures 11-13 probably illustrates these spots with Giemsa stain.

The early schizonts stain a deep blue, are compact, round, and occupy at least half the host cell. The pigment remains granular with a tendency to coalesce (Figs. 14-16). The older and the mature schizonts fill the host cell and produce about 20 merozoites (Figs. 22, 23).

The macrogametocytes take a medium blue stain with a red nucleus, generally eccentric, enclosing a deeper staining irregular area. The pigment, scattered in the cytoplasm, is prominent and rice-grain shaped (Fig. 24). The microgametocyte stains reddish-purple and has a large circular mottled nucleus which may show a deeper staining bar. The pigment is dark to yellowish-brown and sometimes found entirely within a vacuole (Fig. 25).

The parasite, as pointed out by Eyles (1963) has a penchant for invading reticulocytes. Warren *et al* (1966) possessed a greater amount of material, and employed statistical methods to show that the parasite selectively invades mature erythrocytes.

In 1968, Rudzinska and Trager, after studying the fine structure of the parasite and its host cell, were able to show that the trophozoites do not have typical protozoan mitochondria, but they do have a double-membraned organelle which, it is assumed, carries out the functions of the mitochondria. The young parasite feeds on the host cell by pinocytosis, taking in portions of the erythrocytes through invaginations of the plasma membrane or through the cytostome. Digestion of the hemoglobin takes place in small vesicles derived from the food vacuole. The macrogametocytes have two plasma membranes; the inner one thickened in places. The cytoplasm displays Palade's particles, has toxonemes and vesicles of endoplasmic reticulum. The microgametocytes have the whole inner membrane thickened, the cytoplasm displays few Palade's particles and there are no toxonemes.

The host cells with trophozoites are irregularly shaped and show elevated points with knob-like projections and a double membrane. The host erythrocyte has numerous Mauer's clefts which, because they are sometimes continuous with the membranes of the parasite,

PLATE XLV.—*Plasmodium coatneyi*.

Fig. 1. Normal red cell.
Figs. 2, 3, 6, 7. Young trophozoites.
Figs. 4, 5, 8-11. Growing trophozoites.
Figs. 12, 13. Mature trophozoites.
Figs. 14-17. Early schizonts.
Figs. 18-21. Developing schizonts.
Figs. 22, 23. Nearly mature and mature schizonts.
Fig. 24. Mature macrogametocyte.
Fig. 25. Mature microgametocyte.

suggests that they may take their origin from them.

The asexual cycle in the blood occupies 48 hours.

Sporogonic Cycle

PLATE XLVI

Warren and Wharton (1963) were able to infect *A. kochi, A. letifer, A. maculatus, A. sundaicus,* and *A. vagus* but they made no comments on the development of the oocysts. Eyles (1963) in commenting on the sporogonic cycle of *P. coatneyi* reported that no distinguishing characteristics were seen. Subsequently Collins *et al* (1967) reported the infection of *Anopheles b. balabacensis* and *A. freeborni* but only the latter consistently produced sporozoites in the salivary glands. More recently, studies were made to determine the growth rate of the oocysts of *P. coatneyi* in *A. b. balabacensis, A. maculatus,* and *A. freeborni.* The results of these observations were presented in Table 36.

In *A. b. balabacensis,* the oocysts at day 6 had a mean diameter of 19 µ with a range of 12 to 26 µ. The oocysts continued to grow so that by day 11, the mean size was 61 µ with a range of 24 to 90 µ and sporozoites were present in the salivary glands.

In *A. maculatus,* the oocysts appeared to slow down in their rate of growth after day 7. However, oocyst differentiation was seen as early as day 9 and sporozoites, though scarce, were present in the salivary glands on day 12. The mean diameters of the oocysts in *A. maculatus* were considerably smaller on days 8 through 11 than were those of *A. b. balabacensis.*

In *A. freeborni,* the development was apparently normal through day 10. After day 10, there was no evidence of further development; by day 12, many of the oocysts were in various stages of degeneration. No sporozoites were found in the salivary glands although as indicated earlier (Collins *et al,* 1967) low level infections of the salivary glands of *A. freeborni* have been found. An interesting sidelight was that fully developed infections in intact animals, carrying abundant gametocytes, were rarely infectious to mosquitoes. However, once the animals were splenectomized, mosquito infections followed almost immediately with the intensity of the infection in the mosquitoes usually correlated with the 48-hour asexual periodicity (Fig. 62).

A comparison of the mean oocyst diameters of *P. coatneyi* with *P. cynomolgi* (Fig. 63) indicates that *P. coatneyi* is a smaller parasite and it requires one day longer for the sporozoites to appear in the salivary glands.

The sporozoites in *A. b. balabacensis* were shown to be infective; infections were transmitted to 6 *M. mulatta* monkeys by mosquito bites with prepatent periods from 10 to 15 days with a mean of 13.2 days. Dissected salivary glands and triturated bodies *of A. freeborni* mosquitoes infected with *P. coatneyi* were inoculated into 5 *M. mulatta* monkeys. Three of the animals developed an infection with prepatent periods of 14, 15, and 15 days, respectively. We do not know if *A. maculatus* will transmit this parasite although we have seen seemingly viable sporozoites in their glands. Three attempts to transmit the infection by bites of *A. freeborni* mosquitoes to rhesus monkeys have failed.

Cycle in the Tissue

PLATE XLVII

Following the Held *et al* (1967) technique of intrahepatic inoculation of sporozoites, Held and Contacos (1967) carried out a detailed study of the growth stages of *P. coatneyi* in the rhesus monkey. Liver biopsies were done on days 6, 7, 8, 9, 10, and 11 following the introduction of sporozoites; and growth forms for each of the days, except day 11, were described and illustrated in a series of 69 figures. The 6-day forms measured 19 to 22 µ and the oldest forms, i.e., 10-day measured 40 to 48 µ. Different parasites studied on the same day demonstrated the wide extent of heteromorphism in the species which served to confirm their opinion that the tissue stages do not exhibit morphological characteristics which will allow for the separation of species.

Course of Infection

The natural host of *P. coatneyi* is *Macaca irus* (= *fascicularis),* the kra monkey, and in that

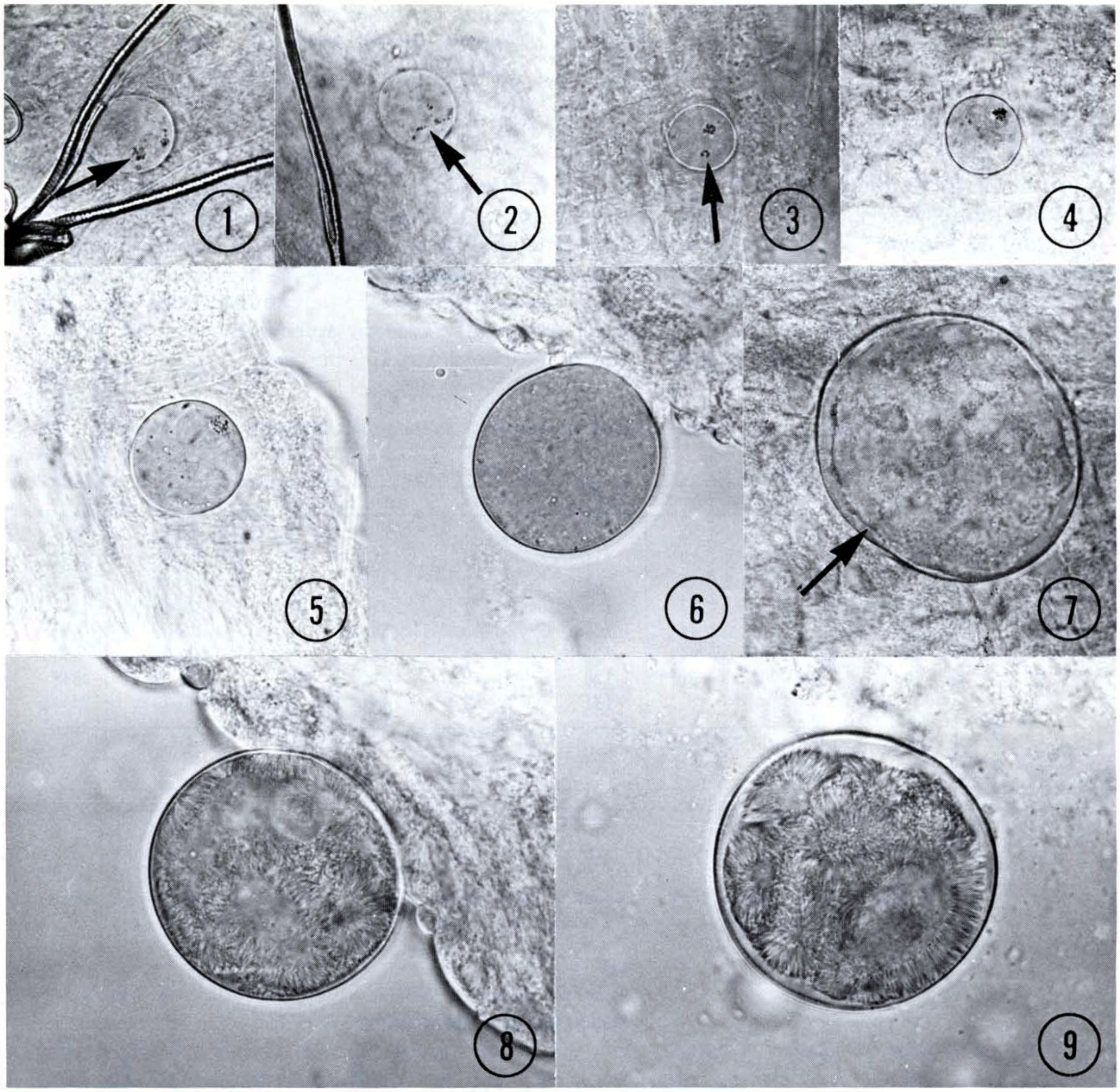


PLATE XLVI.—Developing oocysts of *Plasmodium coatneyi* in *Anopheles b. balabacensis* mosquitoes. X 580.

Fig. 1. 6-day oocyst showing clumped pigment.
Fig. 2. 6-day oocyst showing linear arrangement of pigment.
Fig. 3. 7-day oocyst showing two clumps of pigment.
Fig. 4. 7-day oocyst showing large clump of pigment.
Fig. 5. 8-day oocyst.
Fig. 6. 9-day oocyst.
Fig. 7. 10-day oocyst showing early stages of differentiation.
Fig. 8. 11-day differentiated oocyst.
Fig. 9. Fully differentiated 11-day oocyst showing withdrawal of sporozoite mass from oocyst wall.

animal, the parasite produces a mild low-grade infection that persists for a long time. When the infection is transferred to clean, laboratory-reared *M. fascicularis* by blood inoculation, the peak parasitemias range from 15,000 to 57,000 per mm^3 with the older parasites retreating from the peripheral circulation (Fig. 64).

Other monkeys, *Presbytis cristatus, M. nemestrina,* and *M. speciosa* (= arctoides) were more resistant to infection than M. *fascicularis* (Fig. 64); gibbons, *Hylobates lar,* either refused the infection or allowed it to run a very low course. In each of the hosts, the morphology of the parasite remained unchanged and continued to express the tertian cycle.

In the rhesus monkey, *M. mulatta* (Fig. 65), blood-induced infections may be explosive with peak counts greater than 500,000 per mm^3, resulting in death of a large proportion of the animals (40 percent of our test animals) unless the infection is treated with schizontocidal drugs well ahead of the crisis. Sporozoite-induced infections in intact *M. mulatta* monkeys had a 33 percent mortality rate. The mortality rate in splenectomized *M. mulatta* monkeys was 100 percent.

TABLE 36.—Oocyst diameters of *Plasmodium coatneyi* in *Anopheles b. balabacensis, A. maculatus,* and *A. freeborni.*

Days after Infection	*A. b. balabacensis*			*A. maculatus*			*A. freeborni*		
	No.	Range*	Mean	No.	Range	Mean	No.	Range	Mean
5							114	8-19	14
6	114	12-26	19	111	12-26	21	188	12-30	19
7	125	14-40	25	107	14-40	25	104	14-45	26
8	134	19-60	37	111	20-51	31	101	18-51	34
9	119	17-67	44	134	21-65	39†	140	12-66	41†
10	107	14-74	54†	122	20-54	38†	124	19-80	53†
11	103	24-90	61†**	124	18-70	43†	111	18-78	54†
12				131	26-63	44†**	74	21-90	49†‡
Totals	702	12-90		840	12-70		956	8-90	

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

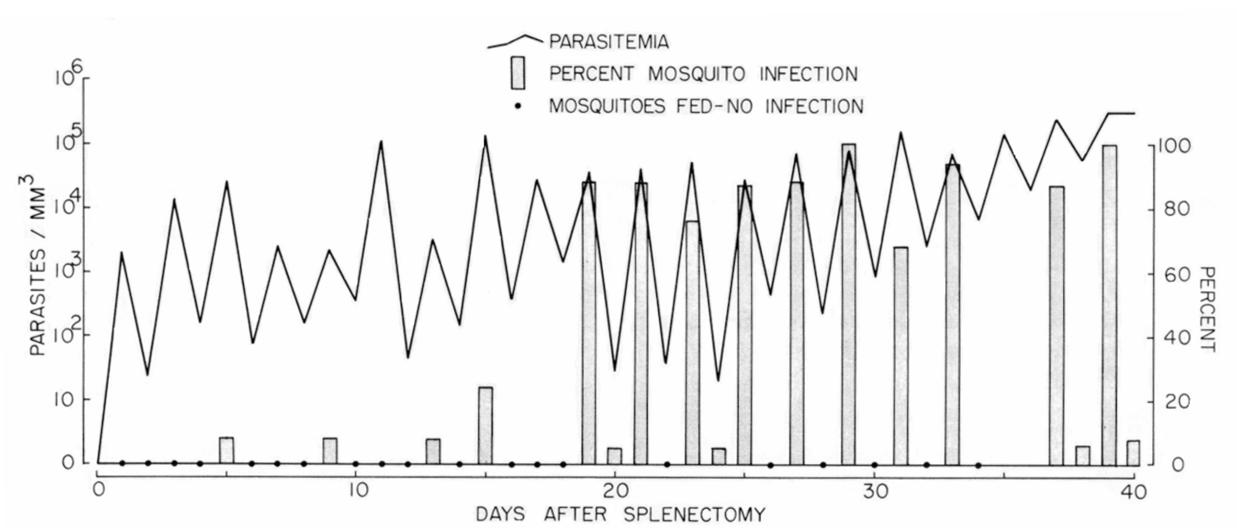


FIGURE 62.—Infectivity of *Plasmodium coatneyi* to *Anopheles freeborni* mosquitoes when fed on a splenectomized *Macaca mulatta* monkey.

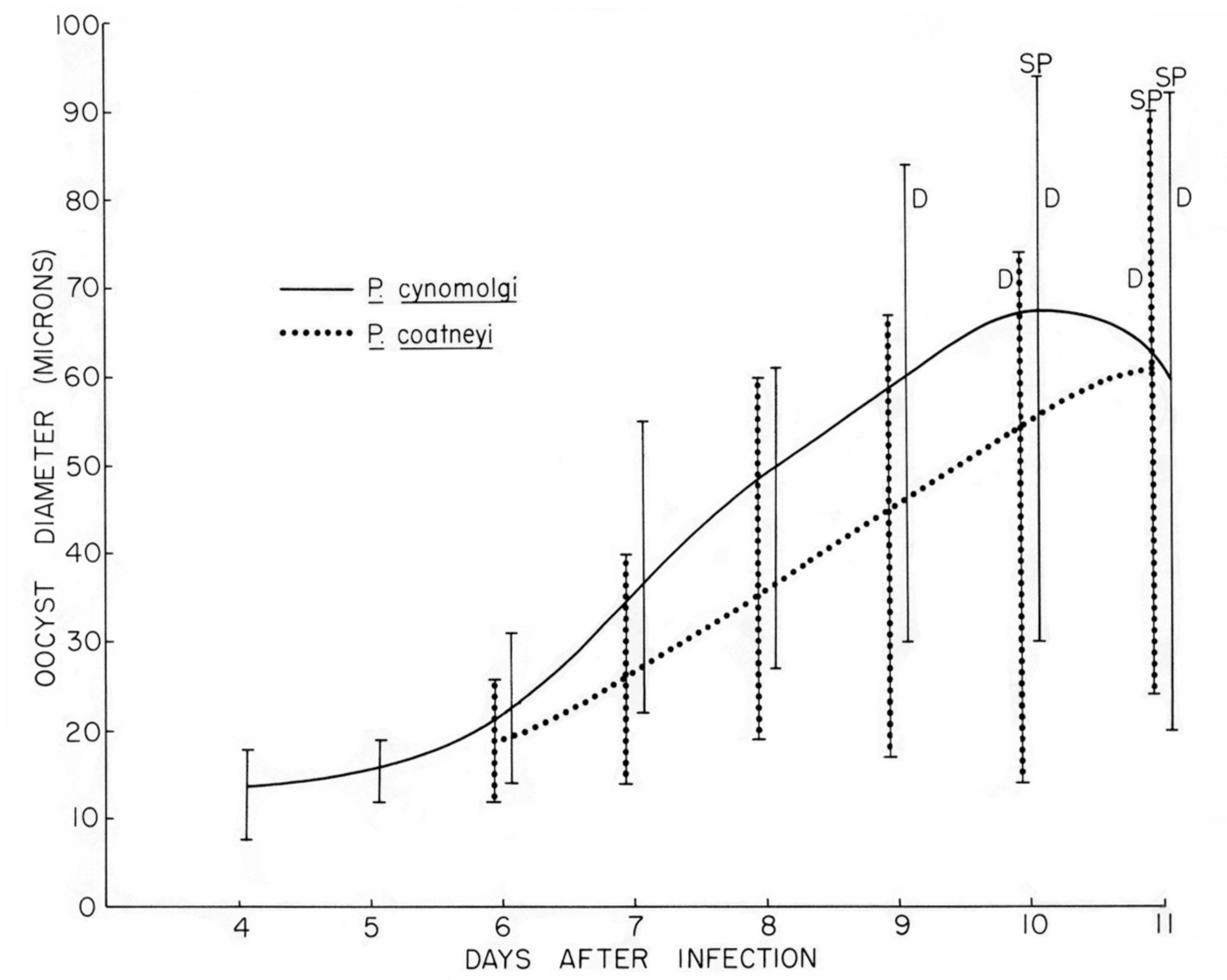


FIGURE 63.—Range in oocyst diameters and the mean oocyst diameter curve of *Plasmodium coatneyi* and *P. cynomolgi* in *Anopheles b. balabacensis* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

Infections induced through the bites of *A. b. balabacensis* in intact rhesus monkeys appear to follow the general pattern of blood-induced infections. Three such animals (Fig. 66) exhibited prepatent periods of 10, 11, and 14 days after which the initial parasitemia climbed rapidly to peak counts of 160,000 to 800,000 per mm^3 between the 7th and 9th days of patent parasitemia only to decline, and then, exhibit a second rise some three weeks later. After the second rise, the parasitemia continued to decline but evidenced the alternate high and low parasite counts during an observation period of 60 days.

Host Specificity

In nature, *P. coatneyi* appears to be limited to the natural host, *Macaca fascicularis,* of peninsular Malaysia and the Philippines (Eyles *et al,* 1962; 1963). The best experimental host is the rhesus monkey, *Macaca mulatta,* which is highly susceptible to infection either by the inoculation of parasitized blood or by sporozoites. Attempts to infect other simian hosts, the silvered leaf coloboid *P. cristatus, M. arctoides,* and *M. nemestrina* have been successful but the infections were all of a low order. Following the discovery of this parasite with its falciparum-like characteristics,

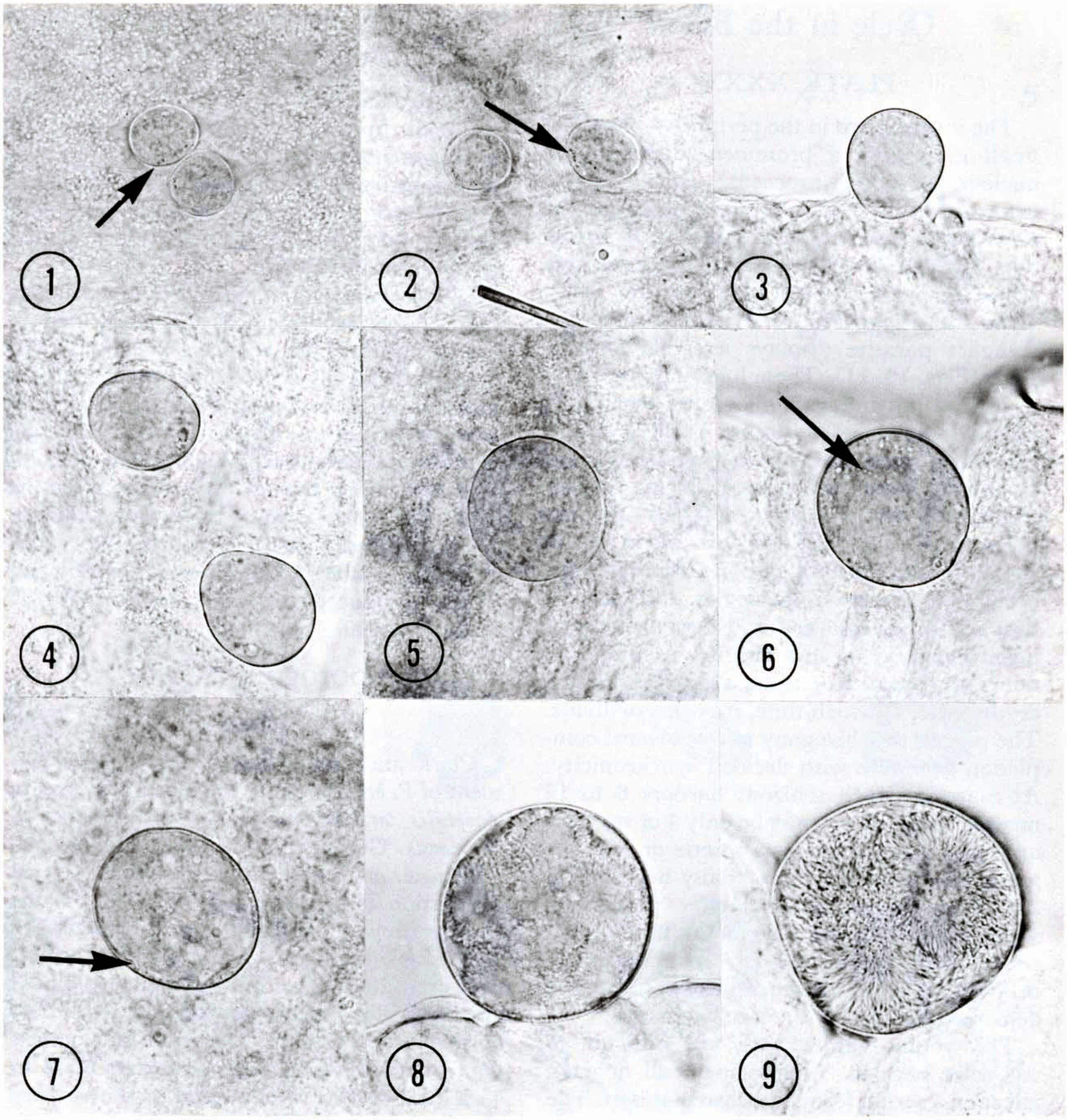


PLATE XLVII.—Exoerythrocytic bodies of *Plasmodium coatneyi* in liver tissue of *Macaca mulatta* monkeys. X 580 (Except Fig. 6).

Fig. 1. 6-day body.
Fig. 2. 7-day body.
Fig. 3. 7-day body showing three prominent vacuoles.
Fig. 4. 8-day body showing abundant large flocculi.
Fig. 5. 9-day body showing abundant, irregular-shaped flocculi.
Fig. 6. 9-day body. X 740.
Fig. 7. 10-day body.
Fig. 8. 10-day body showing two prominent vacuoles.
Fig. 9. 10-day body.

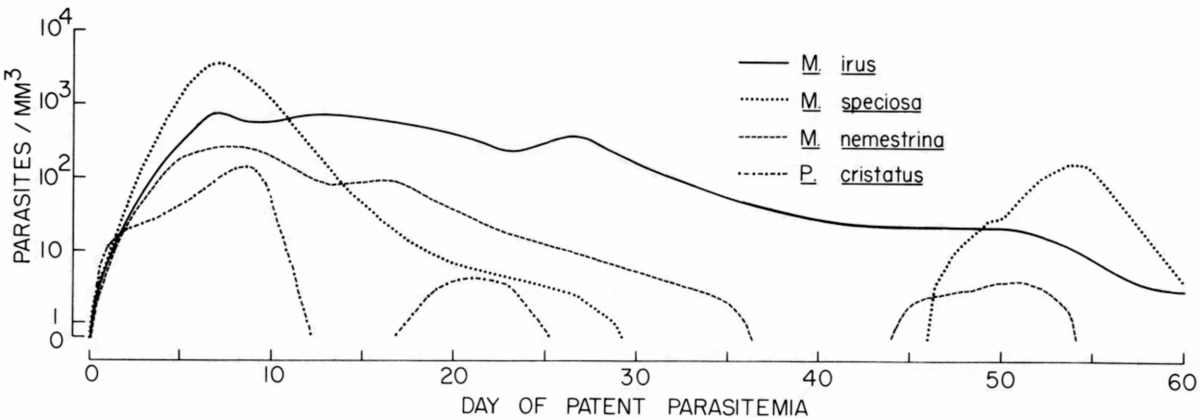


FIGURE 64.—Median parasitemia curves of infections of *Plasmodium coatneyi* in 8 *Macaca irus* (= *fascicularis*), 3 *M. speiosa*, (= *arctoides*), 3 *M. nemestrina* and 3 *Presbytis cristatus* monkeys.

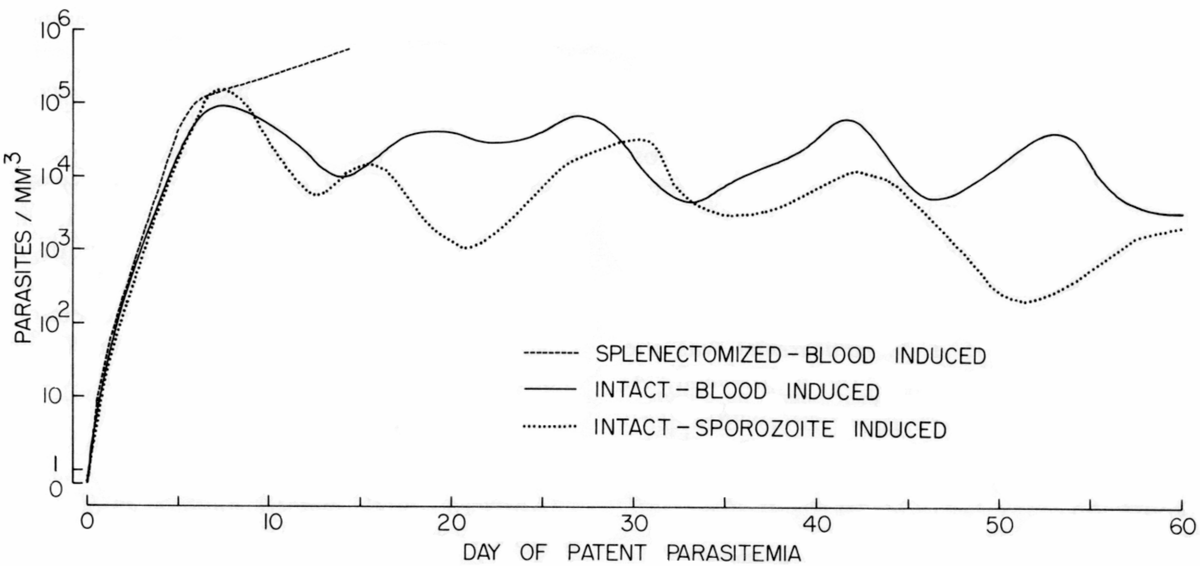


FIGURE 65. Median parasitemia curves of infections of *Plasmodium coatneyi* in 77 intact (66 blood-induced and 11 sporozoite-induced infections) and 6 splenectomized *Macaca mulatta* monkeys.

investigators were anxious to learn if the parasite would express the same characteristics if it became established in man. However, to date all attempts in that direction have failed. Garnham (1965) reported the transfer of parasitized blood from a rhesus monkey to a paretic patient and we (1963 and 1967) made three unsuccessful attempts, over a four-year period, to infect nine volunteers with observation periods extending from 90 to 180 days after biting episodes utilizing *A. freeborni* and *A. b. balabacensis* mosquitoes. It is of interest in this connection that among 6 rhesus monkeys, *M. mulatta,* exposed at the same time as the volunteers, and, to bites of the same mosquitoes, five developed normal patent infections.

Warren and Wharton (1963) were of the opinion, based on finding *P. coatneyi* in *A. hackeri,* that the vector was zoophilic. They were able to obtain infection, through the development of oocysts, in: *A. maculatus, A. kochi, A. sundaicus, A. vagus, A. philippinensis,* and *A. letifer*. More recently we have infected *A.*

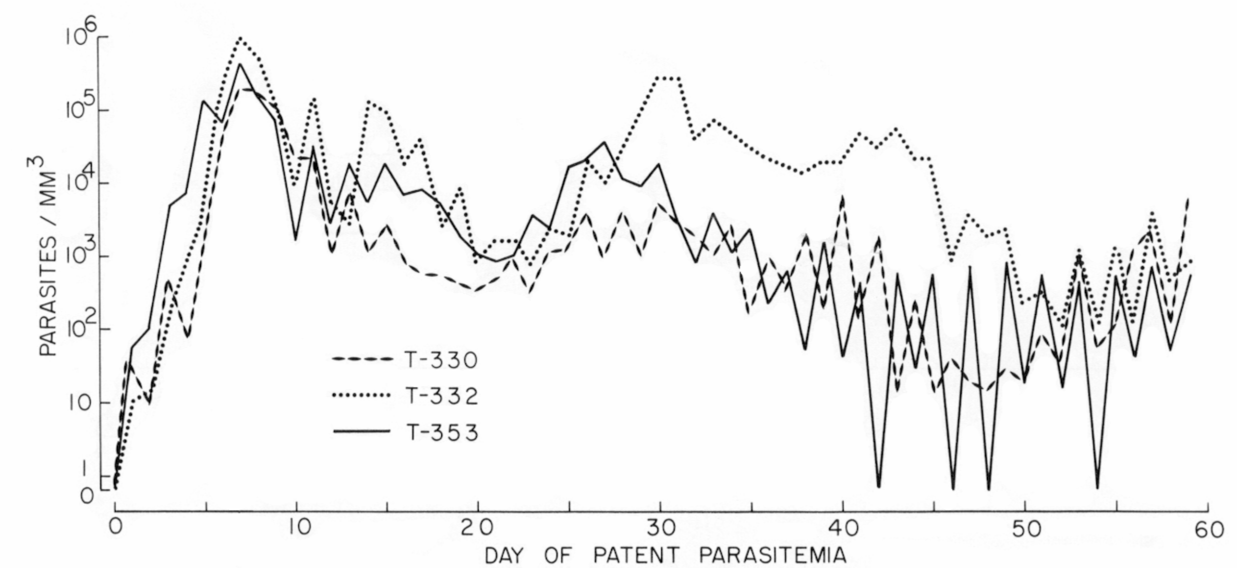


FIGURE 66.—Course of parasitemia in three *Macaca mulatta* monkeys infected with *Plasmodium coatneyi* by sporozoite inoculation.

b. balabacensis, A. freeborni, A. stephensi, A. albimanus, A. atroparvus, A. quadrimaculatus, and *A. maculatus.* Among the 13 species of mosquitoes known to be susceptible, only four: *A. b. balabacensis, A. hackeri, A. maculatus,* and *A. freeborni* have carried the infections to the production of sporozoites in the salivary glands. The susceptibility to infection with *P. coatneyi* varies (Table 37); *A. b. balabacensis* was the most susceptible followed by *A. freeborni, A. maculatus, A. stephensi, A. albimanus, A. atroparvus,* and *A. quadrimaculatus.* The feedings with the other species were too limited to permit proper evaluation.

Immunity and Antigenic Relationships

In order to elucidate some aspects of immunity, Eyles (1963), challenged rhesus monkeys harboring chronic *P. coatneyi* infections with superinfections. These animals were inoculated with parasitized blood of *P. knowlesi, P. inui,* and *P. cynomolgi.* The *P. knowlesi* infections were severe but not fatal indicating some degree of protection; the *P. inui* and *P. cynomolgi* infections were normal indicating no dampening of the infection due to infection with *P. coatneyi.* Voller *et al* (1966) concluded there was considerable cross immunity between *P. knowlesi, P. coatneyi,* and *P. fragile.* However, Voller and Rossan (1969) demonstrated that rhesus monkeys with chronic *P. knowlesi* infections were susceptible to infection with *P. coatneyi.*

Antisera to *P. coatneyi* gave a fluorescent antibody cross-reaction at a very high level to *P. fieldi* (mean reciprocal titer ratio of 100:107) but reacted at a much lower level to other primate malaria antigens (Collins *et al,* 1966), In the reverse procedure, *P. coatneyi* antigen cross-reacted highest to *P. inui* (mean reciprocal titer ratio of 100:57) and at a much lower level to the *P. cynomolgi* and *P. knowlesi* antigens (mean reciprocal titer ratios of 100:27).

TABLE 37.—Comparative infectivity of *Plasmodium coatneyi* to *Anopheles b. balabacensis, A. freeborni, A. maculatus, A. stephensi, A. albimanus, A. atroparvus,* and *A. quadrimaculatus.*

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
Bal						100
Bal : F-1	20	288	389	22.6	47.6	45.7
Bal : Mac	10	93	511	67.7	46.4	15.3
Bal : St-1	8	70	133	35.7	6.0	2.9
Bal : Alb	10	163	144	53.4	1.4	0.4
Bal : Atro	5	60	166	80.0	1.2	0.15
Bal : Q-1	6	62	144	80.6	1.4	0.08

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

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

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