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Plasmodium jefferyi Warren, Coatney, and Skinner, 1966

IN the case of this malaria, as is true of the early history of many others, there is an interesting side-light. In 1964, Dr. A. A. Sandosham, the Director of the Institute of Medical Research in Kuala Lumpur, Malaysia, and the senior author (GRC) were visiting an area in north Malaysia when we chanced to meet a trapper who had, on numerous occasions, obtained animals for us. When queried as to why he had not sent in animals lately, he replied that his traps were in disrepair and that he had no money to pay for wire to fix them. He was given money immediately so his traps could be repaired; and, upon taking our leave, he was told to notify us immediately if he managed to catch a gibbon. The fates were kind. A young female gibbon, G 31, *Hylobates lar,* was brought to the laboratory in July, and when her blood was examined it was found to harbor *Plasmodium youngi* and a low-grade population of another parasite which was morphologically different from any other gibbon malaria. In the hope of obtaining a heavier infection of the then undescribed species, the infection was transferred to a malaria-free gibbon, G 32, by the inoculation of parasitized blood. The original description of the parasite to which Warren, Coatney, and Skinner (1966) gave the name *Plasmodium jefferyi,* in honor of their colleague, Dr. G. M. Jeffery, was based largely on material from that animal.

In reporting on this parasite, the authors were careful to point out the scarcity of young schizonts and the complete lack of mature schizonts in the peripheral blood. They were convinced, however, that they were dealing with a new species and depicted what they saw in a well executed colored plate.

Shortly before the closing of the LPC, NIH laboratory in Kuala Lumpur in 1964, the dual infection, *P. youngi* and the then undescribed species, was passed by inoculation of parasitized blood from G 31 to another gibbon (G 8). Shortly thereafter, both animals were shipped to the Laboratory of Parasite Chemotherapy, Section on Primate Malaria at Chamblee, Georgia. There, it was discovered that G 8 had a high parasite count so blood was withdrawn and deep frozen. Each of the animals died a short time later. The blood was left in the deep freeze until 1968 when a power failure early in that year necessitated immediate action if the specimens were to be saved. The blood was sent to the senior author in New Orleans, Louisiana, and inoculated forthwith into a parasite-free gibbon, *H. lar,* (G 420), at the Delta Regional Primate Research Center, Covington, Louisiana.

The animal (G 420) developed a patent infection 14 days later. However, its infection was quite different from the one in the donor animal (G 8) where *P. youngi* accounted for more than 80 percent of the parasite population at the time blood was drawn for freezing. In G 420, only one species of parasite was present and, although it resembled *P. jefferyi* in many respects, other of its characteristics were quite different. For example, fully mature schizonts, not seen in previous *P. jefferyi* infections, were abundant in the peripheral blood as were distinctive microgametocytes. In addition, the infection appeared to be of a fulminating type.

Our first thought was that maybe the parasite resulted from a latent infection in G 420 triggered by the splenectomy and subsequent

manipulations. The splenectomy had been performed some 40 days prior to the transfer of parasitized blood and the prepatent period was within reasonable limits, that is, 14 days. The idea of a latent infection in G 420 therefore seemed remote; the question was *unde venit,* where did it come from? There was only one possible answer. The parasite had come from the blood of G 8.

That animal, as mentioned earlier, had had a dual infection in which the predominant parasite was *P. youngi* and a low-grade infection with the then undescribed new species (= *P. jefferyi).* The infection in G 420 was not *P. youngi*--that parasite apparently failed to survive the freezing episode. Therefore, the parasite in G 420 was *P. jefferyi* but with characteristics not observed and, consequently, not mentioned in the original description. The situation is understandable when one remembers that the original natural infection exhibited only a low-grade parasitemia with the undescribed species (now carrying the designation *P. jefferyi)* and that that state of affairs was not greatly improved when the infection was transferred to G 32.

The presence of mature schizonts in the peripheral blood of G 420 and a microgametocyte of arresting characteristics had led us away from considering the parasite *P. jefferyi.* Later, the gaps and the inaccuracies in the original description were recognized. Whereupon, Coatney, Orihel, and Warren redescribed the parasite (1969) and included a colored plate to show the complete asexual cycle, the true macrogametocyte, and the distinctive microgametocyte.

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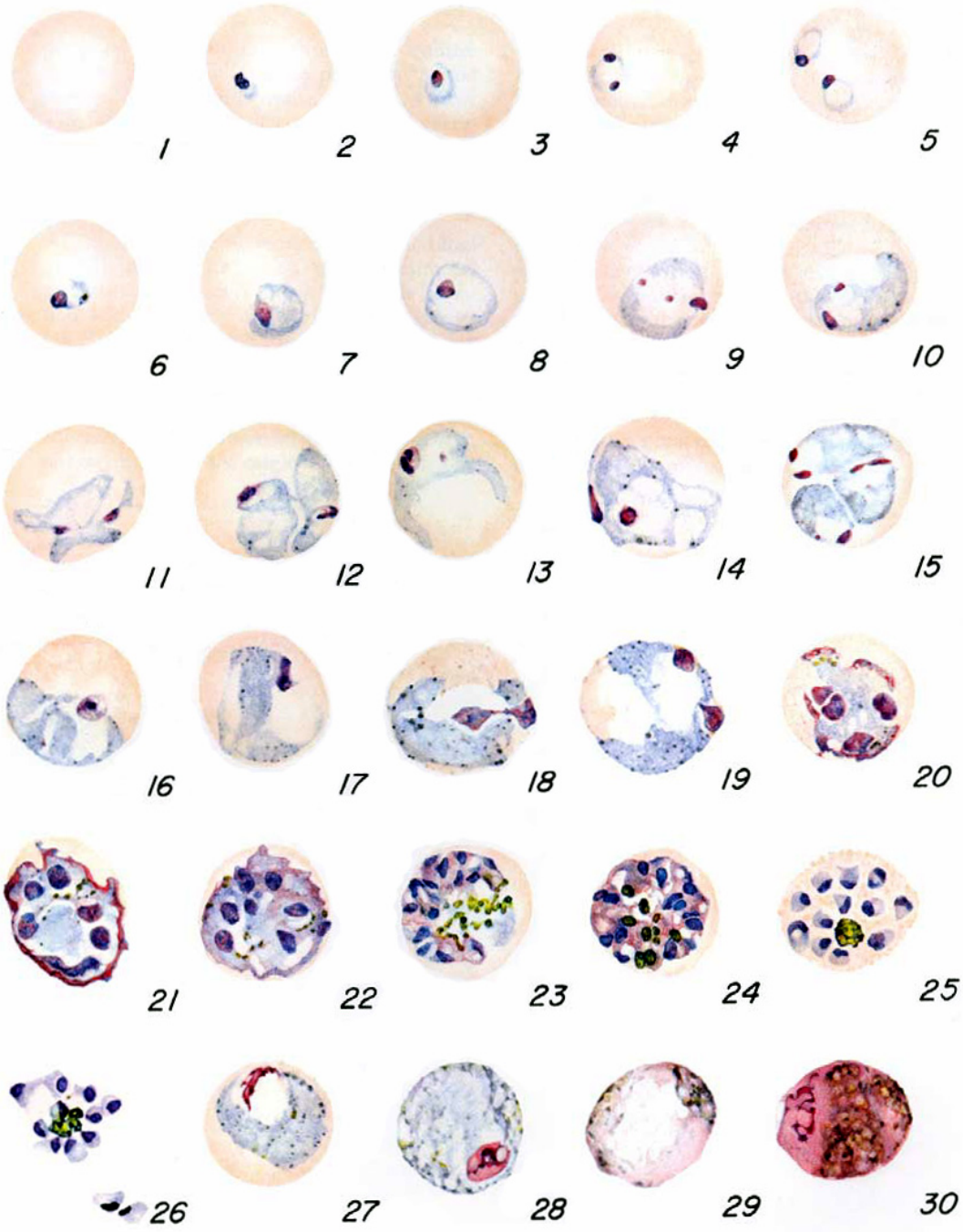


PLASMODIUM JEFFERYI

W. Nicholson

Cycle in the Blood
PLATE XIV

The earliest ring forms display a deep red chromatin dot, measuring 1μ in diameter, sometimes an accessory chromatin dot, and a delicate circle of blue-staining cytoplasm, or there may be two chromatin masses of unequal size. Marginal forms are rare, as are multiple infections in early infections. The host cell is not enlarged (Figs. 2-6). Growth forms may occupy up to one-half or more of the host cell. The nucleus stains a reddish-purple and may lie within a vacuole. The cytoplasm stains a pale blue (Figs. 7-9). The older trophozoites are frequently paisley-shaped with the nucleus, sometimes double, at the broad end and, occasionally, an accessory chromatin dot. The pigment is fine to seed-like and may be arranged along the periphery of the parasite (Figs. 10, 16, 17). Multiple infections of the host cell are common in developed infections (Figs. 5, 12, 14, 15).

Stippling is absent and there is no increase in the size of the host cell. The cytoplasm of the young schizont stains a pale blue and nearly fills the host cell. The pigment is in dust-like granules scattered throughout the cytoplasm. The nuclei stain a deep red (Figs. 18, 19). The older schizonts are more compact and do not fill the host cell. Their nuclei stain a deep red to reddish-purple and number from 4 to 18. The youngest of these forms exhibit a jagged periphery with pale blue cytoplasm confined to the center of the parasite. Light brown pigment granules are distributed unevenly in the cytoplasm (Figs. 20-22). Many of the 6-nucleate forms display an eosinophilic ring reminiscent of *P. fieldi* (Fig. 21). In the older forms, the periphery is smoother, pigment granules are coalesced, massed toward the center of the parasite, and golden brown in color (Figs. 23, 24).

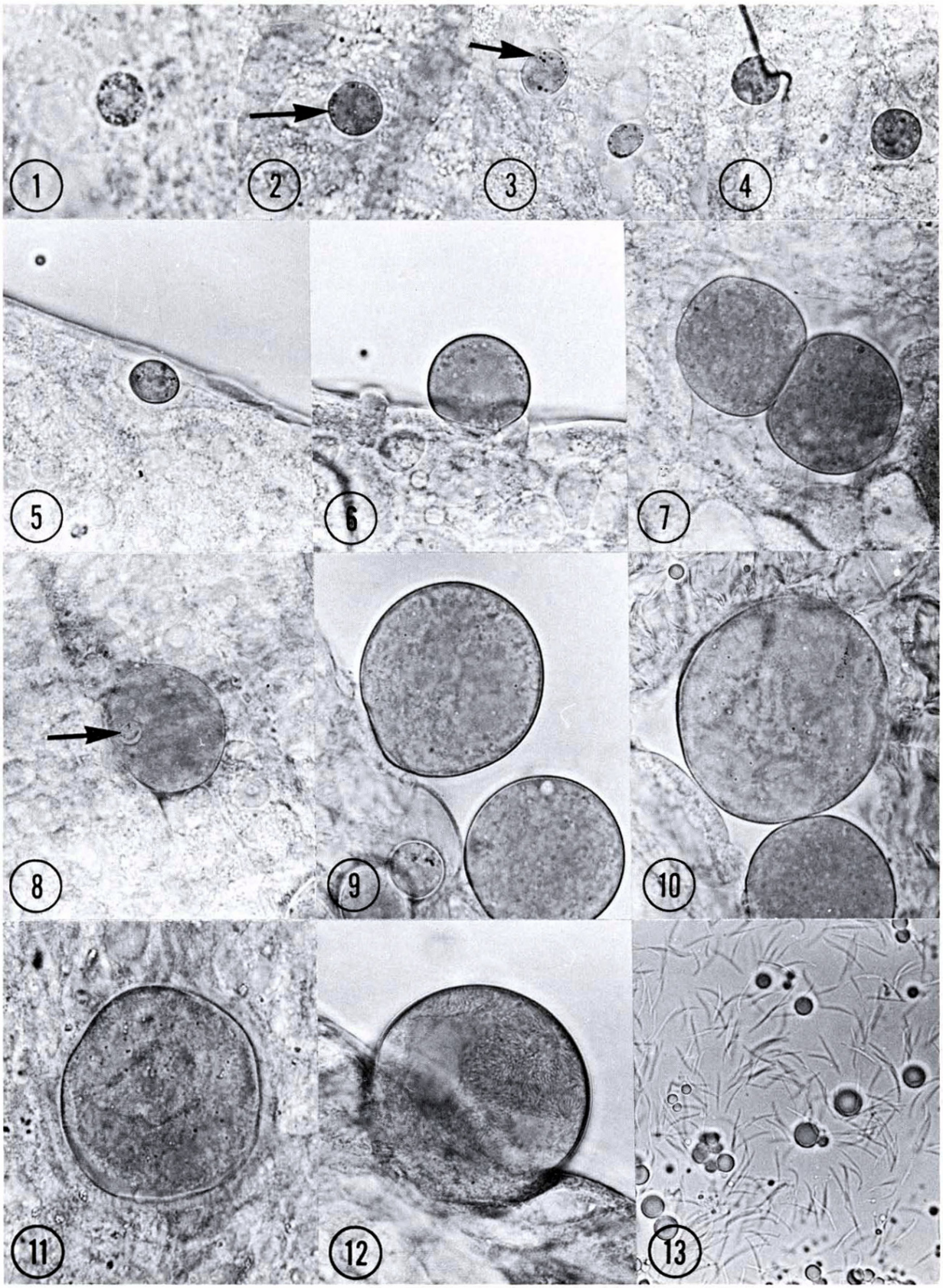
The mature schizonts have a body size less than that of the host cell (Fig. 25) and exhibit 10 to 18 blue-stained nuclei. The gold-black pigment is clumped. The parasite at this stage often assumes bizarre shapes with the merozoites piled on each other.

The young macrogametocytes, 3 to 4 μ in diameter, display a deeply stained red nucleus, with blue cytoplasm and with, or without, a vacuole. Older forms may have one, sometimes two, large vacuoles and fill or almost fill the host cell. The nucleus is bright pink with a deep reddish-purple bar or skein. The cytoplasm is grayish-blue with evenly distributed dust-like pigment (Fig. 27). The adult forms virtually fill the host cell. Their cytoplasm is without vacuoles and stains a light blue; pigment is in greenish-gold granules scattered evenly. The nucleus is bright red with a deep red bar or strand (Fig. 28).

The young microgametocytes, 3 to 4 μ in diameter, have a deeply stained red nucleus, sometimes two nuclear masses, compact cytoplasm, and only a suggestion of a vacuole next to the nucleus; the cytoplasm appears a very light brown. Older growth forms are roughly ellipsoidal, jug-shaped with one side depressed. The cytoplasm is generally without color and with fine dust-like pigment. The nucleus stains a light pink with a red to purple bar or skein (Fig. 29). Adult forms are predominantly oval, sometimes circular, with a slightly ragged appearance. The nucleus is located at the small end of the parasite and stains dark rose with a reddish-purple bar, band, or skein. The cytoplasm stains reddish-pink and is overlaid by a golden-brown bead-like pigment sometimes arranged to present a stocking-cap effect to the more bulbous portion of the parasite.

PLATE XIV.—*Plasmodium jefferyi* from the gibbon.

Fig. 1. Normal red blood cell.
Figs. 2-6. Young trophozoites.
Figs. 7-17. Older trophozoites.
Figs. 18, 19. Young schizonts.
Figs. 20-24. Older schizonts.
Figs. 25-26. Mature schizonts.
Figs. 27, 28. Immature and mature macrogametocytes.
Figs. 29, 30. Immature and mature microgametocytes.
(*Plate reprinted, courtesy of the Journal of Parasitology.*)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The parasite has a 48-hour periodicity.

Sporogonic Cycle
PLATE XV

During the course of the infection of *P. jefferyi* in gibbon 420, mosquitoes were shipped by air from our laboratory in Chamblee, Georgia, to New Orleans, Louisiana, and carried to the Delta Regional Primate Research Center in Covington, Louisiana, where they were allowed to feed on the animal; after feeding they were returned by air. The total travel time did not exceed 30 hours. Upon return, the mosquitoes were held at 25° C for the remainder of the extrinsic incubation period. Beginning on day 5 and continuing through day 14, sample mosquitoes were examined for the presence of oocysts (Collins and Orihel, 1969).

The oocyst diameters of *P. jefferyi* in four species of *Anopheles* are presented in Table 14. In *A. b. balabacensis,* on day five, the oocysts had a mean diameter of 9 μ , with a range of 7 to 11 μ . They continued to grow so that on day 13, the mean diameter was 57 μ , with a range of 33 to 77 μ . Sporozoites were present in the salivary glands on day 13.

In *A. freeborni,* the oocysts grew from a mean diameter of 8 μ, on day 5 to a mean of 45 μ, on day 13. Although differentiation was apparent by day 12, sporozoites were not found in the salivary glands until day 15 and then at a very low level.

In *A. maculatus,* the oocysts grew at a slower pace than in *A. b. balabacensis* and in *A. freeborni.* The mean diameter on day 13 was 30 μ, versus 57 μ for *A. b. balabacensis,* and some of the oocysts had differentiated. Sporozoites were not seen in the salivary glands until day 17 and then at a low level and, in only one mosquito.

In *A. quadrimaculatus,* the growth rate was difficult to determine because of the limited number of oocysts. Oocyst differentiation was present by day 13 but salivary gland infections were not found through 17 days of observation. It thus appears that of the four test mosquitoes only *A. b. balabacensis* was a favorable host for *P. jefferyi.*

A comparison of the growth curve of *P. jefferyi* with that of *P. cynomolgi* in *A. b. balabacensis* mosquitoes (Fig. 30) shows that *P. cynomolgi* is considerably larger and completes its development approximately 3 days sooner than does *P. jefferyi.* A comparison with another gibbon malaria parasite, *P. hylobati* indicates that *P. jefferyi* is much smaller but takes only one day longer for sporozoites to appear in the salivary glands.

As discussed later, it was not possible to determine if the sporozoites were infective. Even though EE bodies were produced after inoculation of these sporozoites into a clean, splenectomized gibbon, no erythrocytic infection resulted.

Cycle in the Tissue
PLATE XVI

Although four species of gibbon malarias are known, exoerythrocytic parasites of

Plate XV.—Developing oocysts and sporozoites of *Plasmodium jefferyi* in *Anopheles b. balabacensis* mosquitoes. X 580 (except Figs. 1 and 2).

Fig. 1 4-day oocyst. X 1300.
Fig. 2. 6-day oocyst showing peripheral pigment. X1300.
Fig. 3. 7-day oocyst showing prominent pigment.
Fig. 4. 8-day oocyst with pigment becoming less distinct.
Fig. 5. 9-day oocyst.
Fig. 6. 10-day oocyst.
Fig. 7. 10-day oocysts.
Fig. 8. 11-day oocyst showing vacuole containing pigment.
Fig. 9. 12-day oocysts showing two with normal development and one which has failed to develop.
Fig. 10. 12-day oocysts, one of which is showing early differentiation.
Fig. 11. 13-day oocyst showing more advanced differentiation.
Fig. 12. 13-day oocyst showing full differentiation.
Fig. 13. Sporozoites from salivary gland tissue 14 days after feeding.

TABLE 14.—Oocyst diameters of *Plasmodium jefferyi* in *Anopheles b. balabacensis, A. freeborni, A. maculatus,* and *A. quadrimaculatus.*

Days after Infection	*A. b. balabacensis*			*A. freeborni*			*A. maculatus*			*A. quadrimaculatus*		
	No.	Range*	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
5	70	7-11	9	101	5-10	8	20	8-11	9	5	7-11	10
6	57	7-17	12	62	6-13	9	31	7-13	10	4	8-14	8
7	95	8-24	14	100	7-18	12	24	9-19	13	8	8-18	12
8	100	9-26	16	54	8-25	14	27	9-21	12	5	15-26	20
9	38	14-27	19	61	8-20	14	35	9-31	17	38	12-37	18
10	100	9-46	24	100	12-40	19	16	12-50	17	18	14-51	28
11	24	13-36	25	100	15-50	30	34	8-36	21	29	11-40	24
12	100	19-73	40†	29	14-59	31†	23	14-44	27	16	17-54	40
13	41	33-77	57†**	76	14-73	45†	56	19-64	30†	4	28-59	46†
14							80	12-61	35†	2	53-55	54†

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

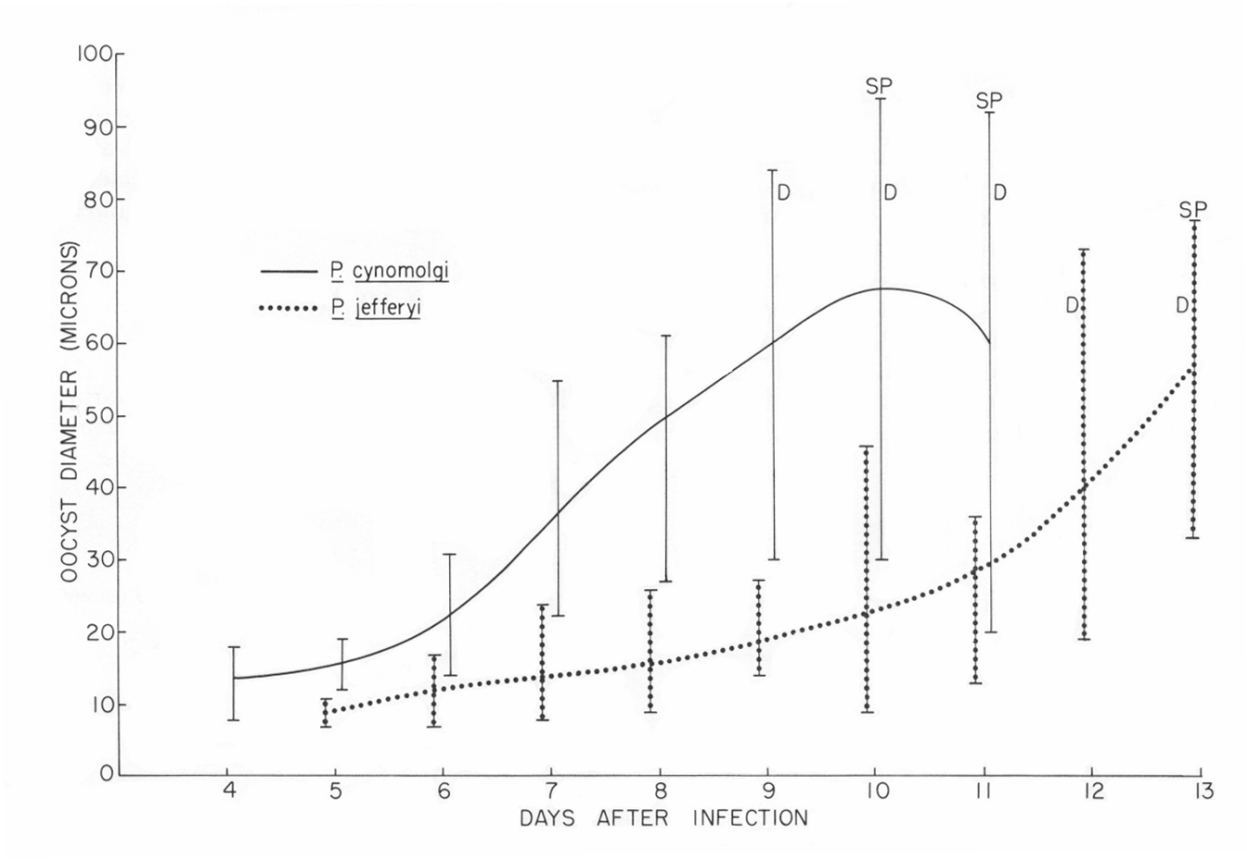

FIGURE 30.—Mean oocyst growth curve and ranges in oocyst diameters of *Plasmodium cynomolgi* and *P. jefferyi* in *Anopheles b. balabacensis* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

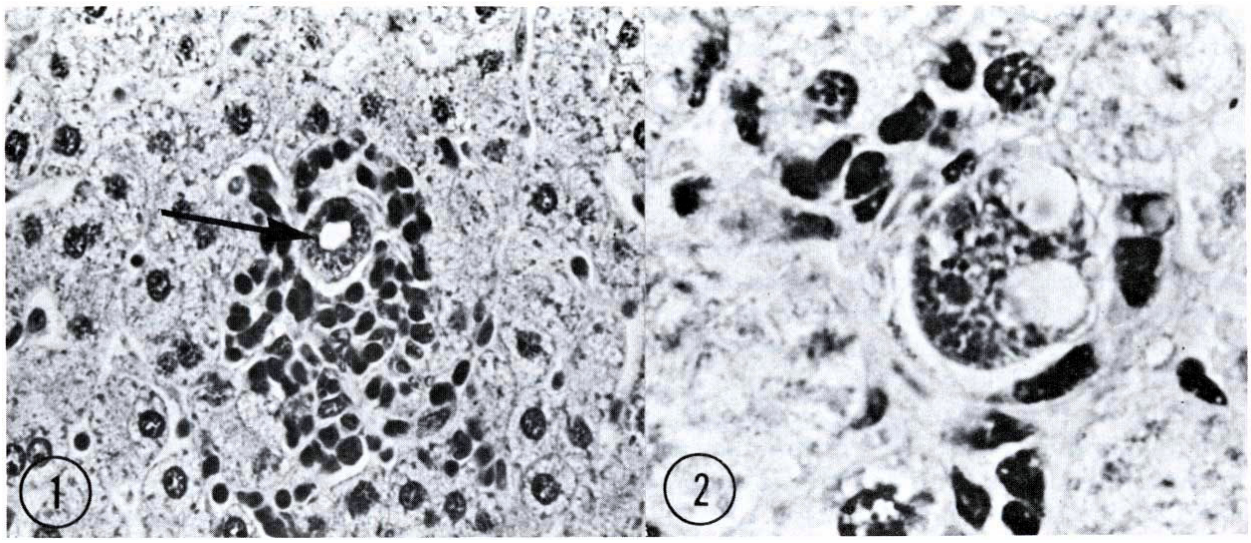

PLATE XVI.—Exoerythrocytic bodies of *Plasmodium jefferyi* in *Hylobates lar*.

Fig. 1. 6-day exoerythrocytic body surrounded by mononuclear cell infiltration. X 580

Fig. 2. 6-day exoerythrocytic body containing two prominent vacuoles. X 930

Plasmodium jefferyi were the first to be described (Sodeman *et al,* 1969). An experimental infection of *P. jefferyi* in a gibbon *(H. lar pileatus)* was induced by inoculating sporozoites directly into the liver at laparotomy, and, by sporozoites introduced intravenously. On day 6, following the inoculations, a biopsy at the site of infection of the liver was taken and the tissue sectioned at 1, 2, 3, 4, and 6μ. Numerous EE bodies of *P. jefferyi* were found in the sections.

The tissue parasites were round to elliptical. The average dimensions were 16.8 by 19.4 μ with a range of 10.8 to 21.6 by 14.4 to 24.0 μ. The edge of the parasite was smooth and usually enclosed by a distinct thin limiting membrane. The nuclei were usually round but frequently appeared diploid; some had bar and triangular shapes. The nuclei stained magenta, were about 1.0 to 1.5 μ in diameter, and did not display a pattern of distribution.

The cytoplasm was granular in texture and stained a pale blue with irregular-shaped, dark blue, aggregates ("flocculi") scattered through the cytoplasm. The aggregates stained homogeneously but in thin sections small holes were evident. A prominent feature of *P. jefferyi* EE bodies was the presence of 0 to 5 large, round to oval, vacuoles in the body, (Fig. 1, 2). These structures, 4.1 by 4.8 μ , often had pink or deep-purple stained material in them.

The EE bodies caused enlargement of the liver cell and pushed the normal sized nucleus to one side. Vacuoles were seldom found in unparasitized liver cells. Focal mononuclear cell infiltrates were scattered through the sections; some were associated with portal regions. Infrequently, EE bodies were found within these infiltrates. Mononuclear cell infiltration was not infrequent in areas surrounding the EE bodies, which suggests that the parasite provoked an unfavorable host response (Fig. 1).

The general morphological features of *P. jefferyi* EE bodies are similar to those of other primate malarias as discussed by Held *et al,* (1967). At that time it appeared that the most consistant feature of a given species was the average size of its EE bodies in relation to the day of infection. However, comparison shows that this is hazardous because their measurements present overlapping ranges. If, with the present data, that criterion is not reliable, and there appear to be no other distinguishing features for recognizing the EE stages of *P. jefferyi,* such as nuclear clefts or cytoplasmic patterns different from those in many other simian malaria species; then, species cannot be separated, at the present time, on the basis of the morphology of the fixed tissue schizonts.

Sodeman *et al* in discussing the presence of the small holes, seen in the 1 to 2 μ sections of the flocculi, mentioned that they might be there to increase surface area. These structures are not mentioned as occurring in the flocculi of other primate malarias, but Sodeman *et al* were able to demonstrate them in thin sections of the EE bodies of *P. cynomolgi* B strain showing that they are not limited to *P. jefferyi* and, therefore, not a character for separating the species. The presence of vacuoles in the *P. jefferyi* material has been reported repeatedly in other species. Held *et al* (1967) felt that they might be fixation artifacts, or that they had a host relationship (Eyles, 1960) or that they are present in the living form and have a respiratory function (Shortt and Garnham, 1948).

It is known that certain drugs may cause vacuoles (Eyles and Coatney, 1962) and, also, vacuoles may occur in degenerating EE bodies (Sodeman *et al,* 1969a). When all aspects of vacuolization in the *P. jefferyi* material were considered it seemed likely that they were due to the process of degeneration, mainly because of the presence of more mononuclear cell infiltrates than usually seen, and, to the fact that the host animal failed to develop a recognizable patent parasitemia; a fact that was discussed earlier. We can say that no drugs were authorized and, to our knowledge, none was given to the animal (G 453) but because it was housed some distance away from the laboratory and not under our immediate control--it could have happened.

Course of Infection

This species of malaria has been observed in only 4 gibbons. In three of them, it was mixed with *P. youngi.* The infections were of a low level with the *P. youngi* parasite predominating (Fig. 31). In splenectomized gibbon 420, however, the infection with *P. jefferyi* reached a high level, (285,000 per mm^3 on the 8th day of patency); the animal became weak and lethargic. Treatment with antimalarial drug was necessary and the animal recovered. Upon recrudescence of the infection, the animal again became ill and very weak, requiring additional antimalarial treatment. It thus appears that *P. jefferyi* in the splenectomized gibbon in the early and/or acute stages may be fulminating in character and may be fatal.

Host Specificity

The only known natural host is the gibbon, *Hylobates lar* from West Malaysia. Attempts to

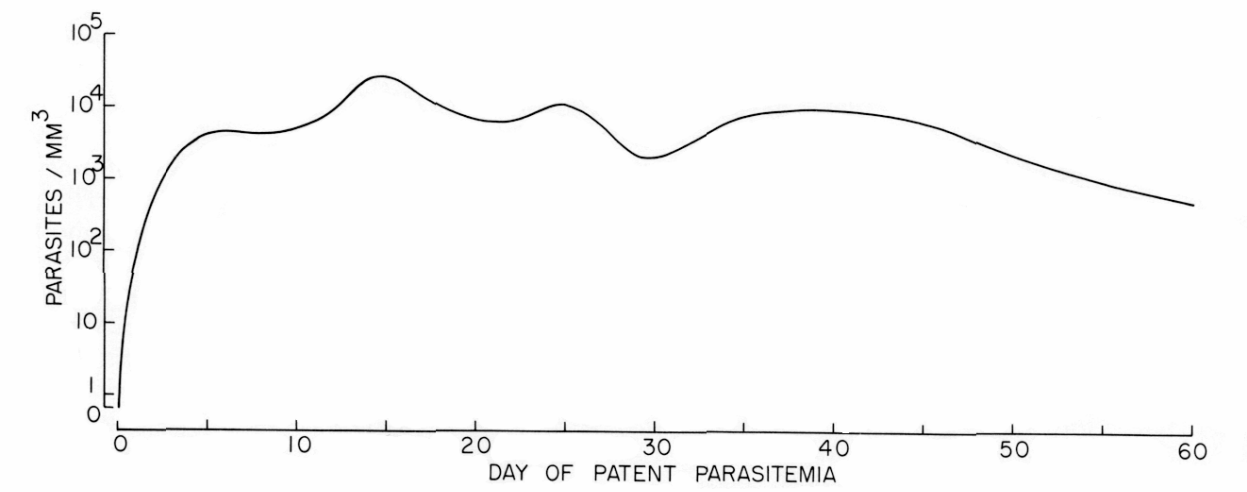

FIGURE 31.—Mean parasitemia curve of *Plasmodium jefferyi* infections in two *Hylobates lar* gibbons infected by the inoculation of parasitized blood.

infect two volunteers by the bites of heavily infected *A. b. balabacensis* mosquitoes were unsuccessful.

The susceptibility of different mosquito species to *P. jefferyi* is based on our single feeding of four species. The comparative susceptibility of these mosquitoes to infection is shown in Table 15. As indicated earlier, however, only *A. b. balabacensis* produced heavily infected salivary glands. The *A. freeborni* and *A. maculatus* mosquitoes had only rare salivary gland infections.

Immunity and Antigenic Relationships

It would appear from the observations on three gibbons that in dual infections with *P. youngi* and *P. jefferyi,* the former is predominant. No additional information is available.

TABLE 15.--Infectivity of *Plasmodium jefferyi* to *Anopheles b. balabacensis, A. freeborni, A. maculatus,* and *A. quadrimaculatus.*

Species	No. positive/ no. dissected	Oocysts/gut	Oocysts/pos. gut
A. b. balabacensis	16/16	53.9	53.9
A. freeborni	21/22	38.1	39.9
A. maculatus	60/79	4.4	5.8
A. quadrimaculatus	36/86	1.5	3.5

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