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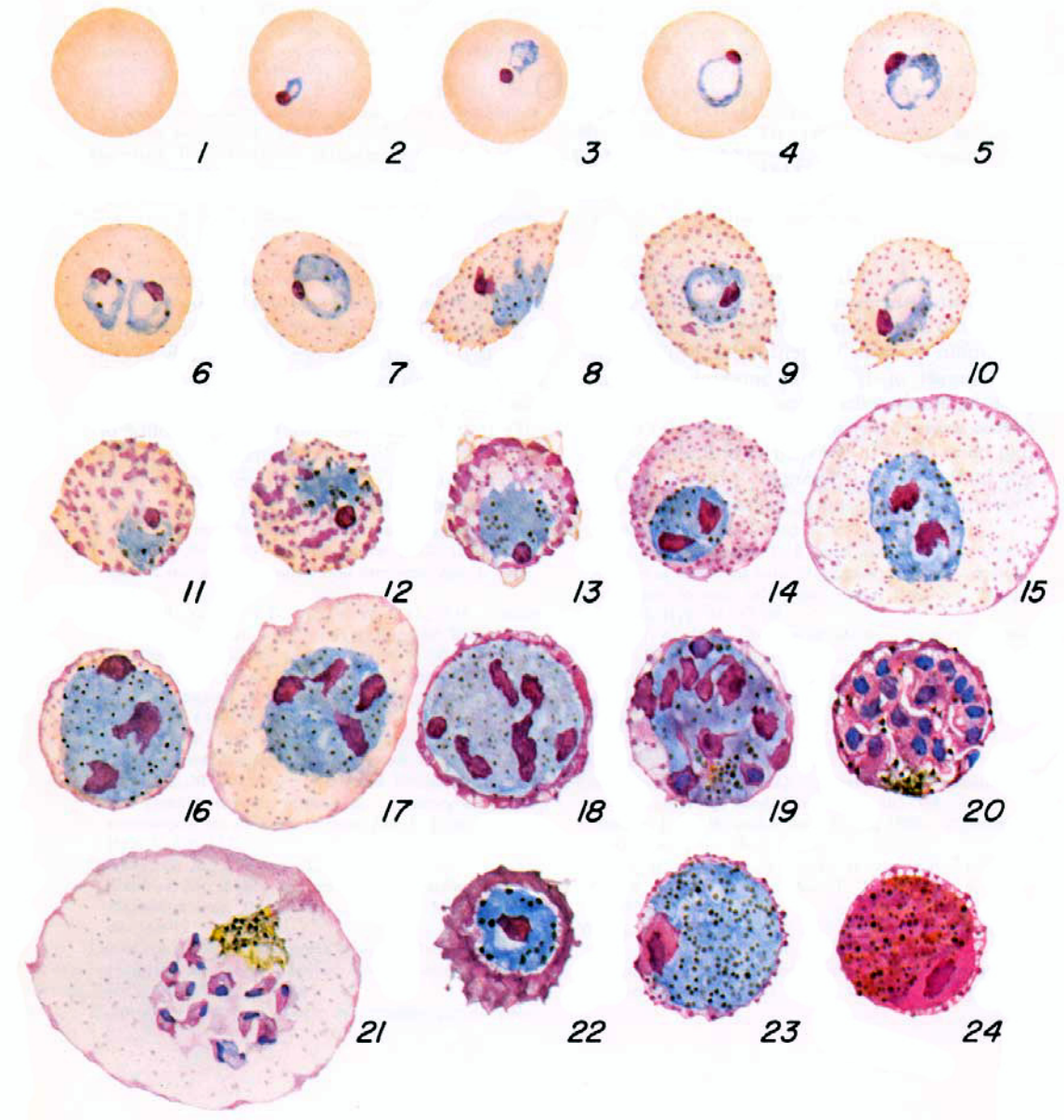
Plasmodium fieldi Eyles, Laing, and Fong, 1962

ONLY three species of malaria were known from macaques when Dr. Eyles embarked on his extraordinary series of studies on simian malaria in peninsular Malaysia in mid-August of 1960. In September of that year, he purchased a young pig-tailed macaque *(Macaca nemestrina)* from a trapper who said it had been taken in the district of Kuala Selangor in the state of Selangor, Malayasia. The monkey was carrying a malaria parasite but the parasitemia was too low to allow for species identification. The animal was splenectomized in January, 1961, after which the parasitemia increased permitting more careful study of the parasite which confirmed an earlier assumption that it was a new species. The Eyles group gave it the name *Plasmodium fieldi* in honor of Dr. John W. Field, who has made outstanding contributions to our knowledge of malaria in general and especially in Malayasia.



PLASMODIUM FIELDI

G. H. Nicholson

0 10μ

Cycle in the Blood
PLATE XXVII

The youngest parasites are ring-shaped and about 3 μ in diameter. Some have double chromatin bodies. Multiple infections of the erythrocyte are not common; stippling appears when the trophozoite is about half-grown (Fig. 5).

The older trophozoites (Figs. 11-13) are compact, rounded or oval, and display very little amoeboidity. The cytoplasm is compact, stains a deep blue, and the nucleus a deep red; pigment is dark and made up of fine grains; Schüffner-type stippling takes a deep red stain. Some host cells are oval-shaped (Figs. 7-9); older parasites, with the vacuole diminished or lost, occur in cells with aggregates of dark eosinophilic masses sometimes larger than their nuclei (Figs. 11-13). The host cell is slightly enlarged.

Immature schizonts (Figs. 14-20) exhibit dense blue-staining cytoplasm and relatively large deep red nuclei; pigment is granular, well distributed, generally black; stippling is heavy, and, as schizogony proceeds, the eosinophilic masses come together to form a deep red border around the developing schizont (Fig. 18). The host cell may be appreciably enlarged--ballooned-out--, some of them assume an oval shape rather than circular (Figs. 15, 17). The mature schizonts (Figs. 20, 21) produce 4 to 16 large merozoites with a mean number of 12. The golden brown pigment forms a large mass ofttimes in the center of the schizont. The host cell may become greatly distorted (Fig. 21 and earlier); the explanation for this is not known but it appears to be distinctive for this parasite.

The adult macrogametocytes have the nucleus placed off-center; it stains dark red. The cytoplasm stains a deep blue, and supports delicate, dark pigment granules scattered in the cytoplasm. The host cell, which may be slightly enlarged, encloses a red ring of coalesced eosinophilic stippling (Fig. 23).

The mature microgametocytes fill the host cell and exhibit a dark pink cytoplasm. The reddish stained nucleus, with a deep red bar-like mass, is located eccentrically (Fig. 24). The pigment granules are heavy and fairly evenly distributed in the cytoplasm. The host cells show pronounced stippling and some exhibit fimbriated edges.

The asexual cycle is 48 hours.

Sporogonic Cycle
PLATE XXVIII

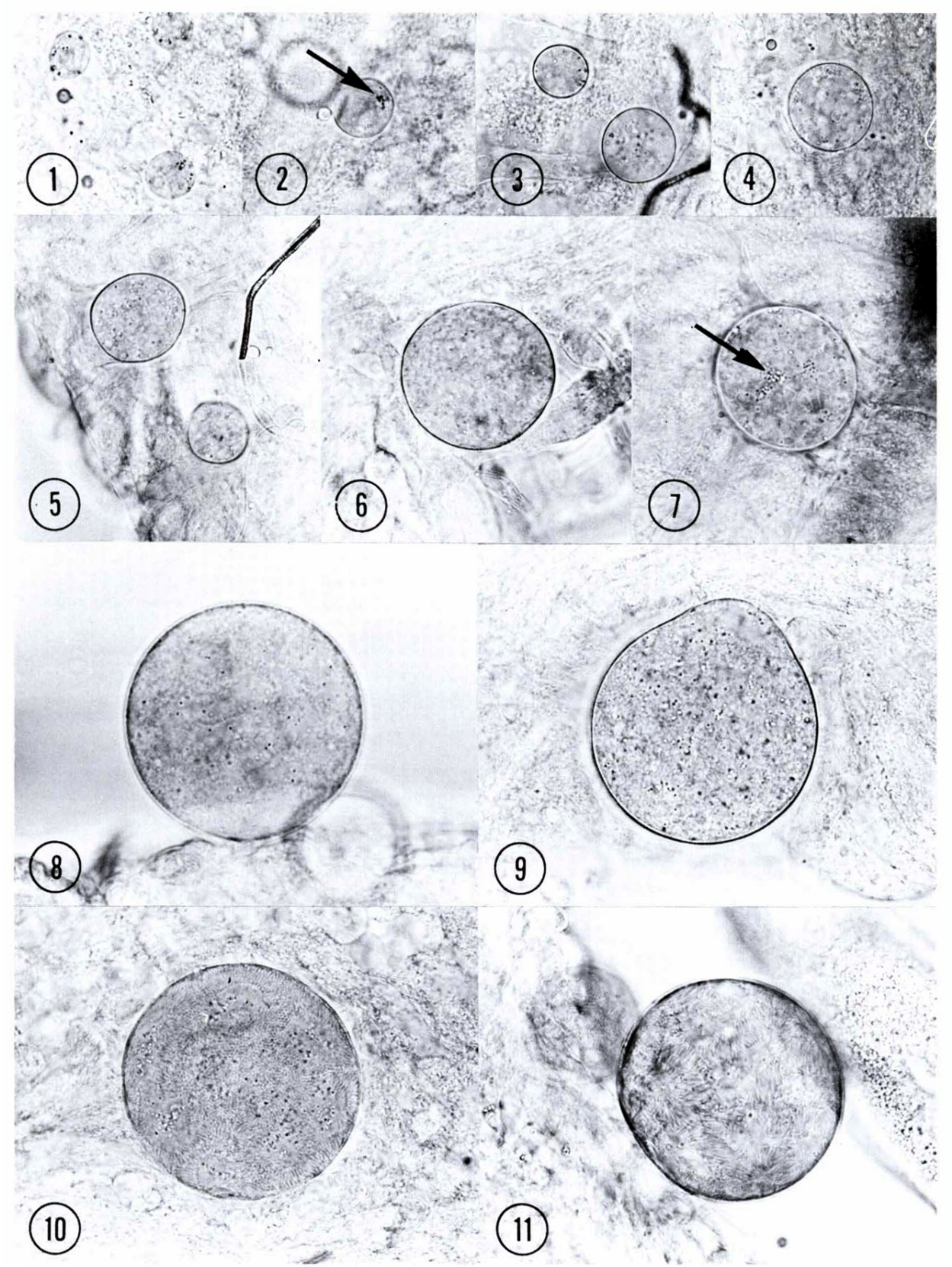
The sporogonic development of *P. fieldi* has been examined in *A. b. balabacensis, A. maculatus,* and *A. freeborni* mosquitoes (Table 23). In *A. b. balabacensis,* at day 5, the mean diameter was 13 μ, with a range of 8 to 14 μ. The oocysts continued to grow so that by day 13, the mean size was 68 μ, with a range of 32 to 96 μ. Sporozoites were present in the salivary glands by day 14.

Although the oocyst measurements in the *A. maculatus* mosquitoes were limited in number, it appeared that the mean diameters were smaller than in the *A. b. balabacensis* during the period of oocyst differentiation. Sporozoites were present in the salivary glands of these mosquitoes by day 14. The oocyst measurements in the *A. freeborni* were within the ranges of those seen in the other two. Although oocyst differentiation appeared to be normal, sporozoites were found only near the dissected guts of the mosquitoes and there was no evidence that they had invaded the salivary glands.

A comparison of the *P. fieldi* oocyst growth

PLATE XXVII.—*Plasmodium fieldi.*

Fig. 1. Normal red cell.
Figs. 2-4. Young trophozoites.
Figs. 5-10. Growing trophozoites.
Figs. 11-13. Nearly mature and mature trophozoites with pronounced eosinophilic stippling.
Figs. 14-19. Developing schizonts showing typical host cell distortion.
Figs. 20, 21. Mature schizonts showing 'ballooned-out' host cell distortion.
Figs. 22, 23. Developing and mature macrogametocytes.
Fig. 24. Mature microgametocyte.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curves with *P. cynomolgi* in *A. b. balabacensis* mosquitoes (Fig. 39), shows that *P. fieldi* requires more time to complete its development, than does *P. cynomolgi.* The oocyst diameters of the *P. cynomolgi* at day 10 were approximately equal to those of *P. fieldi* on day 13. In addition, the appearance of *P. fieldi* sporozoites in the salivary glands required 4 days longer than the *P. cynomolgi* parasite.

In some ways, *P. fieldi* is similar to *P. simiovale* in its sporogonic development. However, during the extrinsic incubation period of 8 to 13 days, oocysts of *P. fieldi* were slightly smaller than those of *P. simiovale.* Also, the latter parasite completed its cycle 1 day sooner. It was shown by Bennett *et al* (1966) that there are differences in the growth rate and time of the appearance of sporozoites in the salivary glands between different sub-species and isolates of *P. cynomolgi.* It is possible that this minor difference between the sporogonic cycles of *P. fieldi* and *P. simiovale* indicates a close relationship between these two species, almost on the same level as those found by Bennett *et al,* between isolates of *P. cynomolgi.*

We obtained transmission of *P. fieldi* to the rhesus monkey via the bites of *A. b. balabacensis* (10 times), by *A. maculatus* (once), and by *A. stephensi* (once) (see Collins, *et al* 1968). In addition, infections have been obtained in our laboratory by the intravenous and/or intrahepatic inoculation of sporozoites from *A. b. balabacensis* (7 times), *A. freeborni* (twice), and *A. stephensi* (twice). The prepatent periods for the 23 infections ranged from 9 to 18 days with a mean of 12.4 days. Coombs *et al* (1968) also obtained transmission of this parasite to the rhesus monkey by the intravenous inoculation of sporozoite from *A. b. balabacensis* mosquitoes. The prepatent period was 13 days.

Our attempts to transmit the infection to man (10 volunteers) via the bites of infected

TABLE 23.—Oocyst diameters of *Plasmodium fieldi* in *Anopheles b. balabacensis, A. maculatus,* and *A. freeborni* mosquitoes.

Days after Infection	*A. b. balabacensis*			*A. maculatus*			*A. freeborni*		
	No.	Range	Mean*	No.	Range	Mean	No.	Range	Mean
4							39	8-14	11
5	59	8-14	13				84	9-18	13
6	62	11-22	16				112	9-19	15
7	115	12-27	19	4	18-30	23	118	11-37	21
8	136	12-35	25	16	15-31	23	126	13-34	24
9	132	17-45	32				97	17-44	30
10	51	18-65	40	55	24-59	44	119	15-59	34
11	59	31-64	52†	31	31-74	49	95	24-72	47
12	160	17-92	59†	144	23-83	50†	15	26-71	53†
13	187	32-96	68†	101	18-79	50†	67	34-83	59†
14	135	25-104	64†**	109	30-85	57†**	23	30-68	50†
Totals	1096	8-104		460	15-85		895	8-83	

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

PLATE XXVIII.—Developing oocysts of *Plasmodium fieldi* in *Anopheles b. balabacensis* mosquitoes. X 580 (Except Figs. 1 & 2).
Fig. 1. 5-day oocysts. X 740.
Fig. 2. 6-day oocyst showing clumped pigment. X 740.
Fig. 3. 7-day oocyst.
Fig. 4. 8-day oocyst.
Fig. 5. 9-day oocyst.
Fig. 6. 10-day oocyst.
Fig. 7. 11-day oocyst showing some small vacuoles.
Fig. 8. 12-day oocyst.
Fig. 9. 13-day oocyst showing numerous small vacuoles.
Fig. 10. 13-day differentiating oocyst.
Fig. 11. 13-day fully differentiated oocyst.

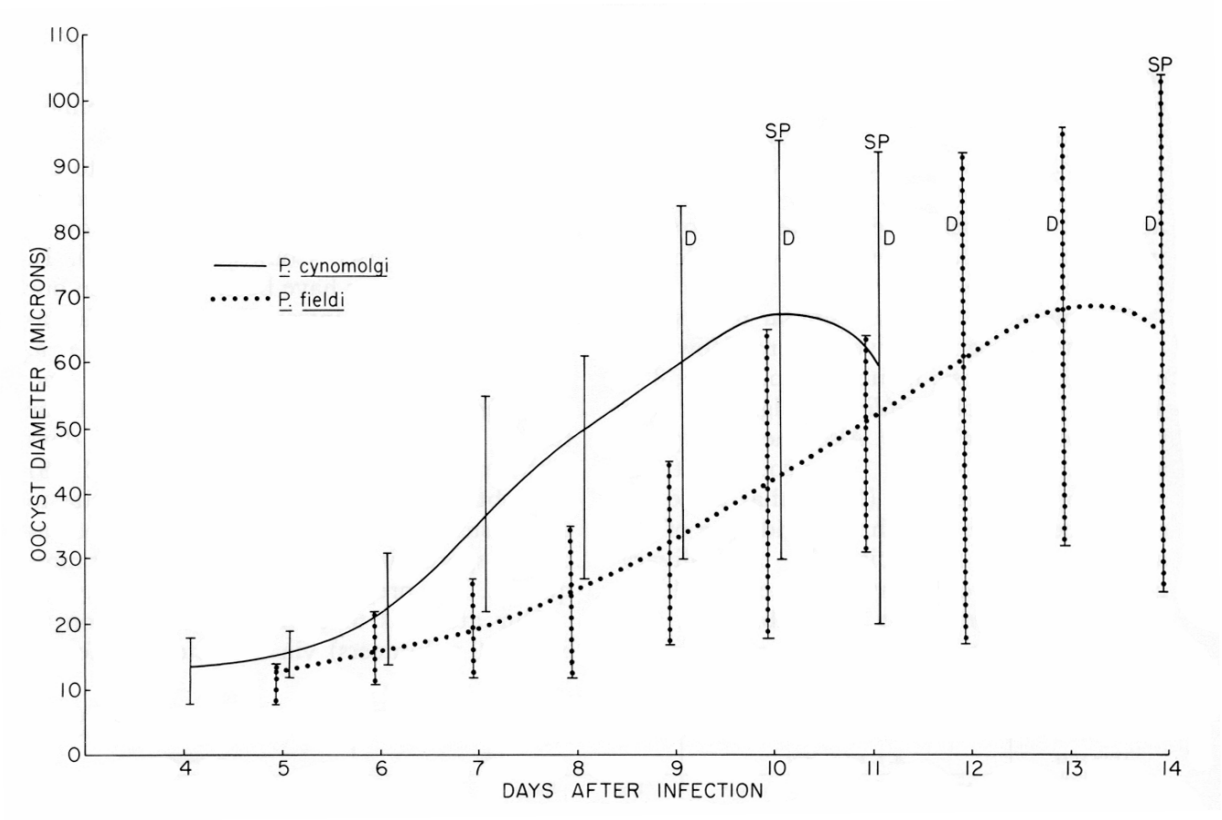

FIGURE 39.—Range in oocyst diameters and mean oocyst diameter curve of *Plasmodium fieldi* and *P. cynomolgi* in *Anopheles b. balabacensis* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

mosquitoes were unsuccessful. The sporozoites were viable because control rhesus monkeys became infected.

Cycle in the Tissue

PLATE XXIX

With the exception of *P. cynomolgi,* it is doubtful if the tissue phase of any simian malaria parasite has been studied more than has *P. fieldi* in the rhesus monkey (Held *et al,* 1967; Coombs *et al,* 1968; and Sodeman *et al,* 1970).

Held and his co-workers described the 8-, 10-, and 12-day forms and their illustrations are models for future investigators to emulate. Generally, the exoerythrocytic bodies were circular to elliptical in section, the body edges were smooth, although some exhibited slight indentations giving the impression of scalloping. The nuclei, 0.5 to 1.0 μ in diameter, stained magenta and were generally circular; some forms suggested a diploid or tetrad configuration. The cytoplasm took a pale blue stain and, in some sections, showed dark blue cytoplasmic aggregates up to 3 μ in diameter. Certain of the exoerythrocytic bodies stained a deeper blue than others which suggested that they were more compact. Some sections exhibited pale pink staining vacuoles up to 3 μ in diameter. In many preparations, there was a partial separation, measuring 1 to 4 μ, between the parasite body itself and the surrounding host tissue which was interpreted as shrinkage following fixation.

Coombs *et al* (1968) supplied data on the 6- and 12-dayforms of the parasite. Two 6-day old forms were spheroid in shape and measured 18 and 20 μ in maximum length. One complete 12-day form was ovoid in shape and measured 40 to 60 μ which agrees with the 12-day form described by Held *et al.*

Sodeman *et al* (1970) studied the 5, 6, 7, and 9-day forms and they agree with the opinion of Held *et al* to the effect that the tissue stages of

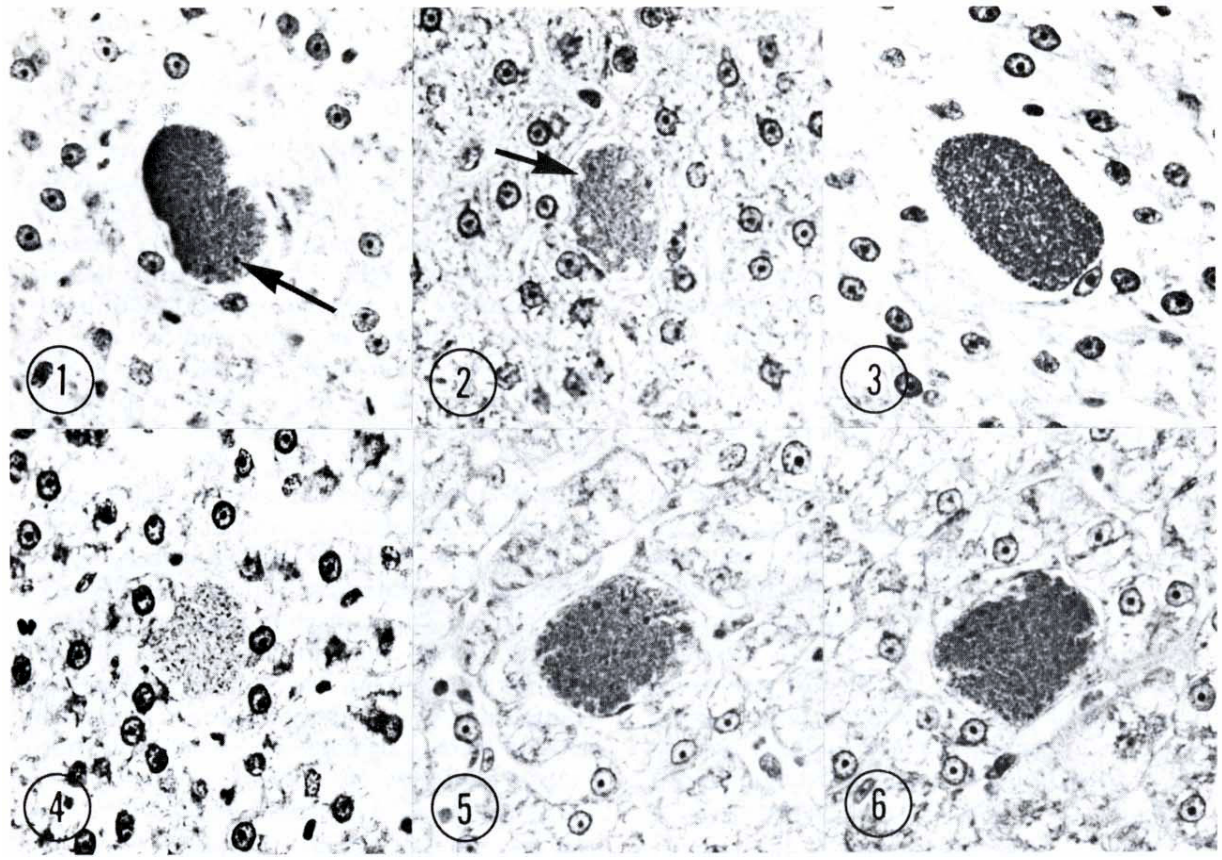

PLATE XXIX.—Exoerythrocytic bodies of *Plasmodium fieldi* in liver tissue of *Macaca mulatta* monkeys. X 580.
Fig.1. 8-day body showing small flocculi.
Fig. 2. 8-day body shwing vacuoles.
Fig. 3. 10-day body.
Fig. 4. 10-day body.
Fig. 5. 12-day body.
Fig. 6. 12-day body.

P. fieldi exhibit no morphological characteristics which would separate them from the other species of *Plasmodium.*

Course of Infection

The course of the natural infection in the original pig-tailed macaque was followed by Eyles *et al* (loc. cit.) for some 3 months, prior to splenectomy, during which it exhibited a low parasitemia ranging from zero to 33 parasites per mm^3 of blood. After splenectomy, the parasite count increased rapidly to some 9,000 parasites per mm^3 of blood and then declined, to persist at a low level for several weeks. When the parasite was introduced into the intact natural host (*M. nemestrina*), the parasite count did not exceed 737 per mm^3 of blood during an observation period of one month. After the animal was splenectomized, the parasite count reached a peak of 196,000 per mm^3 21 days later, after which it declined. Two pig-tailed macaques, splenectomized prior to receiving the infection via inoculation of parasitized blood, developed counts of 51,000 and 127,000 per mm^3 after which their parasitemias declined. Each of the animals continued to show a patent infection, with low to moderate counts, for months.

The same group of workers induced infections with *P. fieldi* in two intact and two splenectomized rhesus *(M. mulatta)* monkeys by the inoculation of parasitized blood. The intact animals displayed parasitemias of 11,000 to 20,000 per mm^3 of blood while, in the splenectomized animals the peak counts ranged from 50,000 to 100,000 per mm^3 of blood.

A summary of our studies with *P. fieldi* (Fig. 40) shows that the parasitemias in blood-induced infections, in intact *M. mulatta* monkeys, reached a peak of approximately 9,000 per mm^3 by day 7 and declined rapidly to a level of approximately 500 per mm^3 by day 15. This level was maintained for the next 30 days, after which, the parasitemia receded slowly to minimal levels. In the splenectomized *M. mulatta* monkeys, the median peak parasitemia was almost 73,000 per mm^3. There was considerable variation in the parasitemia curve in these animals with four separate peaks of parasitemia during the 60-day observation period. At 60 days, the median parasite count was less than 500 per mm^3. In the monkeys infected by sporozoites, the peak parasitemia was the same as in the blood-induced series, but was delayed by about 3 days. The parasitemia then rapidly declined to a minimal level by day 30. The secondary rise to a peak of approximately 100 per mm^3 by day 42, possibly represents relapse activity or the appearance of a new antigenic variant. In the 6 *M. nemestrina* monkeys infected by inoculation of parasitized blood, the peak of the median curve was 475 per mm^3 which obtained on day 9. Although there was a secondary rise in the parasitemia at approximately day 25, the levels were generally minimal.

The phenomenon of relapse had intrigued malariologists even before Thayer (1899) published his series of lectures with illuminating references to relapse in *vivax* malaria. Since then, a prodigious literature has accumulated which can not be gone into here except to point out that the phenomenon has received only cursory examination among the simian malarias. True relapses, in contrast to recrudescences, do occur in sporozoite-induced *P. cynomolgi* infections. Our studies with *P. fieldi* lead us to consider it related to *P. ovale,* a 'relapser' in man, and a life-pattern study was set up to test its relapse potential. Each of seven rhesus monkeys with sporozoite-induced infections was allowed to experience an initial parasitemia, which was treated early, as was each succeeding attack, with either quinine sulfate, at a dosage of 300 mg daily for 5 or 7 days, or chloroquine phosphate 150 mg (base) daily for 2 days or 50 mg daily for 3 days (see Fig. 41). These dosages in our hands were known to be curative of blood-induced infections, and would, on that basis, eradicate the blood forms in the sporozoite-induced infections under study. As may be seen by perusal of Figure 41, each infection exhibited two or more relapses at varying intervals. The infection in one animal (T 688) exhibited 14 relapses during a period of 12 months. The relapses did not fall into a distinct pattern, which was not unexpected, but one may

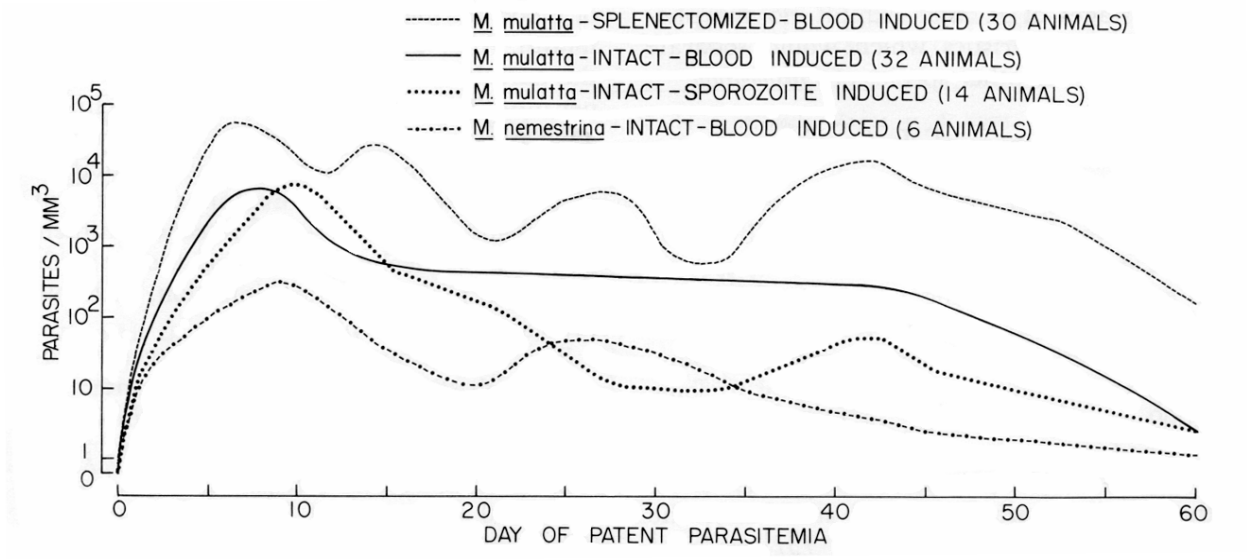

FIGURE 40.—Median parasitemia curves of *Plasmodium fieldi* as seen in 76 *Macaca mulatta* and in 6 *M. nemestrina* monkeys.

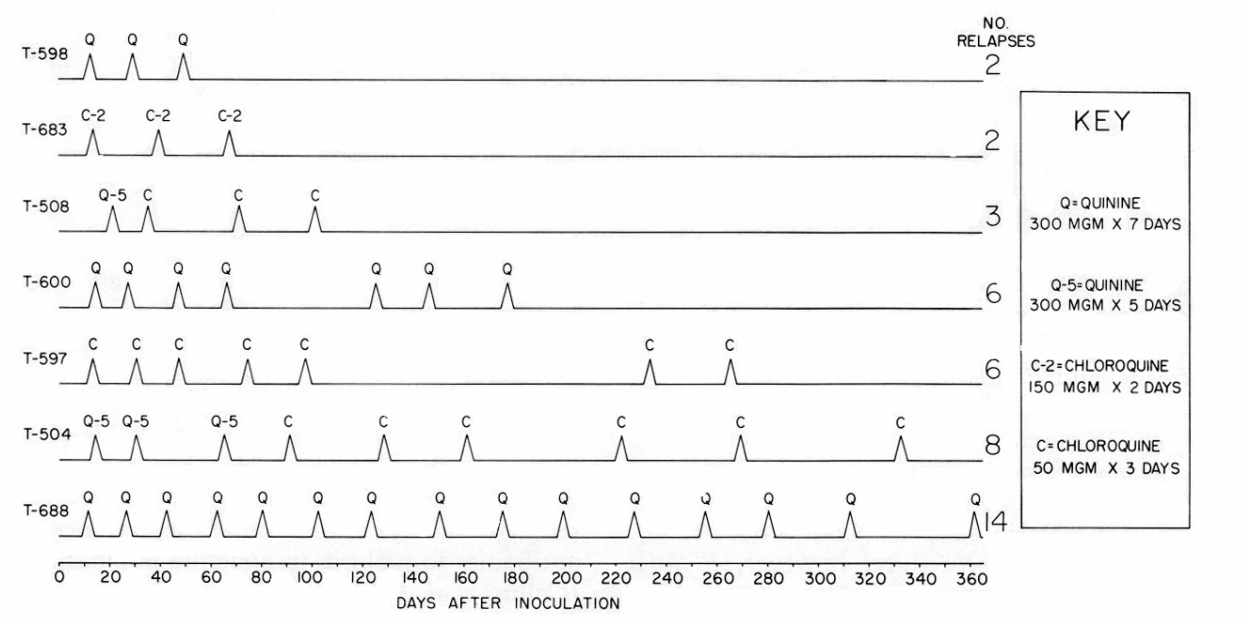

FIGURE 41.—Relapse pattern of *Plasmodium fieldi* as seen in seven *Macaca mulatta* monkeys.

note, that as the time from initial infection increased the tendency for longer intervals between relapses increased also, which *was* expected. The main point was answered, namely, that *P. fieldi* does relapse and that relapse producing infections may last for at least one year.

Host Specificity

The type host of *P. fieldi* is *M. nemestrina,* from which a single isolation was made by Eyles *et al* (1962a). If the parasite was looked for more carefully in this host, it probably would be found, as it was in the kra monkey *(M. irus* (= *fascicularis)*) by Warren and Wharton (1963) in one of twenty of these animals taken in the Kuang forest, north of Kuala Lumpur, Malaysia.

The parasite will also grow in rhesus monkeys *(M. mulatta)* but as Warren *et al* (1964) pointed out the parasite's unique staining characteristics, i.e., large eosinophilic masses and an intense red ring around the parasite, are modified in *M. fascicularis* and in *M. mulatta.* However, these hosts do display the enlarged parasitized host cell. Low level infections have been obtained by us in the baboon *(Papio doguera)* and in *M. radiata.*

Plasmodium fieldi has been isolated from two members of the Leucosphyrus group of mosquitoes, *Anopheles hackeri* and *A. balabacensis introlatus* (Warren and Wharton. 1963). Warren and Wharton (1963) reported that *A. donaldi, A. freeborni, A. hackeri, A. letifer, A. maculatus,* and *A. philippinensis* were susceptible to infection, all at a low level. Additionally, *A. b. balabacensis, A. kochi, A. vagus, A. sinensis, A. albimanus, A. argyropus, A. peditaeniatus, A. atroparvus,* and *A. quadrimaculatus* were shown to be susceptible, at least to the production of oocysts. *Anopheles freeborni* was the most susceptible (Table 24) followed by *A. b. balabacensis, A. kochi,* and *A. vagus.* However, *A. freeborni* did not readily support *P. fieldi* infections to completion and therefore *A. b. balabacensis* is considered the best experimental vector.

Immunity and Antigenic Relationships

Two *M. mulatta* monkeys infected with *P. fieldi* were allowed to have patent parasitemia for 32 and 33 days, respectively, with peak parasitemias of 15,100 and 21,300 per mm^3. They were then given curative treatment with chloroquine. When their blood was parasite-free, one animal was blood-inoculated with *P. fragile* and the other with *P. cynomolgi.*

Neither animal displayed any evidence of immunity since their peak counts reached 5/100 and 11/100 RBC, respectively. In a reverse study, a rhesus monkey was allowed to experience a patent infection with *P. cynomolgi* for 62 days. It was then treated, as above, and later challenged with *P. fieldi.* The fieldi infection was higher than normally expected, a peak count of 89,000 per mm^3. On the basis of these limited data, it appears that there is no cross-immunity among *P. cynomolgi, P. fragile,* and *P. fieldi.*

Antisera to *P. fieldi* gave a fluorescent antibody cross-reaction at only a low level to *P. cynomolgi* (mean reciprocal titer ratio of 100:31) and at much lower levels to other primate malaria antigens (Collins *et al,* 1966). In the reverse procedure, however, *P. fieldi* antigen cross-reacted at a consistently high level. Mean reciprocal titer ratios against the different antisera were as follows: *P. inui,* 100:107; *P. shortti* (= OS strain *P. inui),* 100:47; *P. brasilianum,* 100:24; *P. cynomolgi,* 100:76; *P. coatneyi,* 100:107; *P. gonderi,* 100:41; *P. fragile,* 100:93; and *P. knowlesi,* 100:87.

Plasmodium fieldi antigen has also been shown to react at a high level to *P. malariae, P. falciparum,* and *P. ovale* antisera (Collins *et al,* 1966a; Meuwissen, 1966, 1968). It appears, therefore, that the *P. fieldi* antigen contains a fraction which is common to most, if not all, of the primate malarias. Because of this phenomenon, the *P. fieldi* antigen has been used with human antigens successfully in several serologic surveys to determine the presence of antibodies to malaria in endemic populations (Collins *et al,* 1967, 1968a).

TABLE 24.—Comparative infectivity of *Plasmodium fieldi* to *Anopheles b. balabacensis, A. freeborni, A. kochi, A. vagus, A. maculatus, A. sinensis, A. albimanus, A. argyropus, A. atroparvus, A. peditaeniatus, A. stephensi,* and *A. quadrimaculatus.*

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
Bal						100
Bal : F-1	71	1495	1498	40.5	50.7	129.9
Bal : Kochi	2	42	11	38.1	27.3	55.4
Bal : Vagus	2	28	4	92.9	75.0	39.8
Bal : Mac	16	273	247	49.8	15.2	16.5
Bal : Sin	2	26	13	92.3	53.8	6.3
Bal : Alb	4	113	89	54.0	4.5	4.8
Bal : Arg	1	17	13	88.2	100.0	4.1
Bal : Ped	3	30	20	90.0	25.0	3.6
Bal : Atro	15	337	347	54.6	8.4	3.1
Bal : St-1	10	234	199	53.4	7.6	1.8
Bal : Q-1	17	375	406	52.3	3.9	1.4

* Bal = *Anopheles b. balabacensis,* F-1 = *A. freeborni,* Kochi = *A. kochi,* Vagus = *A. vagus,* Mac = *A. maculatus,* Sin = *A. sinensis,* Alb = *A. albimanus,* Arg = *A. argyropus,* Atro = *A. atroparvus,* Ped = *A. peditaeniatus,* St-1 = *A. stephensi,* and 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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