

Plasmodium inui Halberstaedter and von Prowazek, 1907

AT the same time that Halberstaedter and von Prowazek were studying the parasite of the orangutan, they came across parasites of malaria in *Macacus cynomolgus* (= *Macaca fascicularis*) from Java and in *M. nemestrina* from Sumatra and Borneo. Because the new parasite was different from the one in the orangutan, they wrote a brief description of it, accompanied by illustrations. They named it *Plasmodium inui*, taking the species name from the old generic name of the host, *Inuus*.

It is not unlikely that Mathis and Leger (1911) saw the same parasite in some macaques from Indochina listed as *M. mulatta* and *M. lasiotis tcheliensis*. Bray (1963) believed the hosts were actually *M. assamensis*.

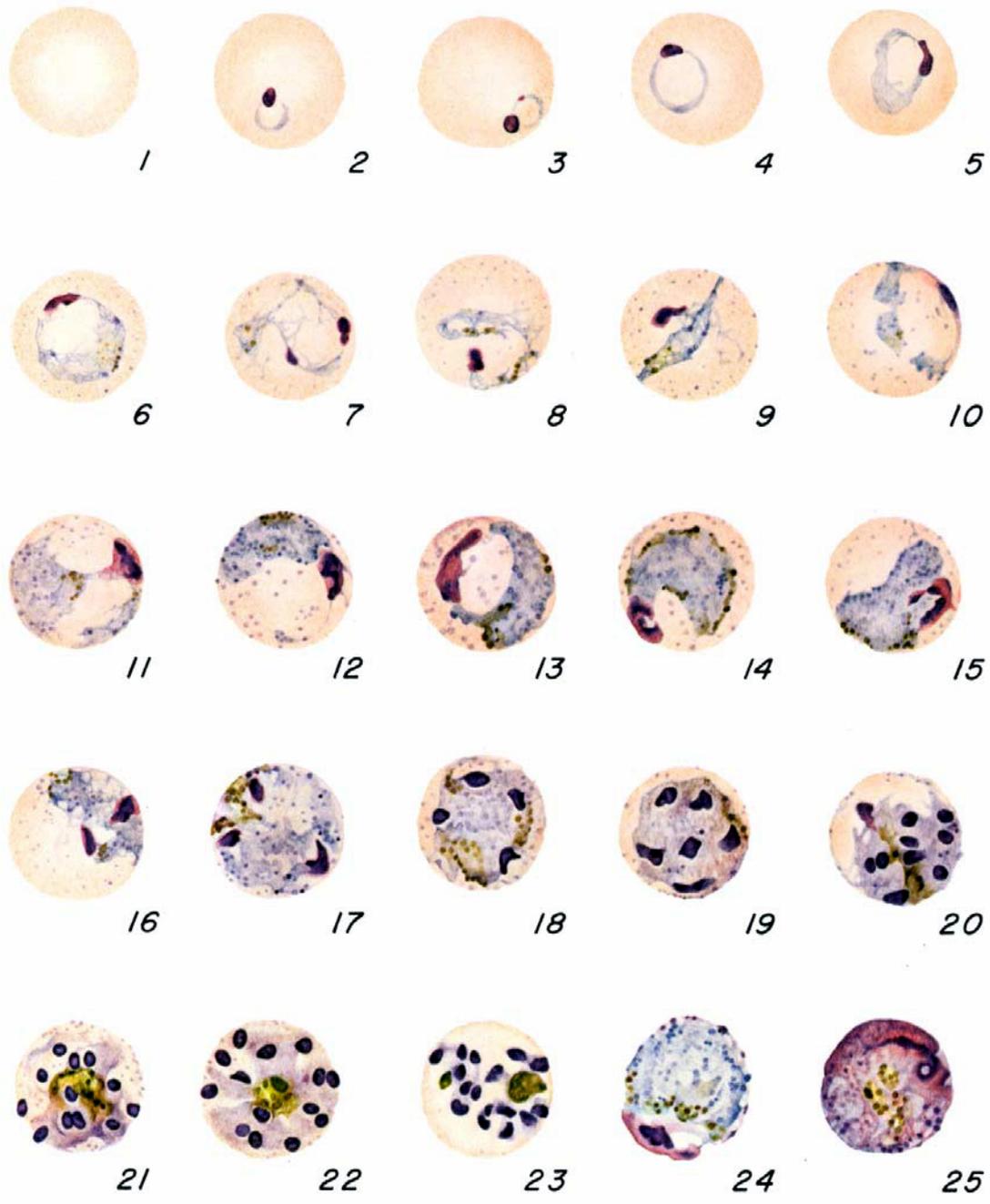
Leger and Bouilliez (1912) found a plasmodium in *M. cynomolgus* (= *M. fascicularis*) which they described briefly. Bouilliez later (1913) published a short paper on the same material. In 1913, Leger and Bouilliez gave a detailed description of the parasite and identified it as *P. inui* Halberstaedter and von Prowazek. They believed it was the same parasite as *P. cynomolgi* Mayer, 1907. In all

probability, the above authors, especially Leger and Bouilliez (1913), were dealing with a mixed infection because they demonstrated a 48-hour cycle, which, of course, could not have been *P. inui*.

Noguchi (1928) reported finding *P. inui* in an *M. cynomolgus* (= *M. fascicularis*) which he had splenectomized in connection with work on Bartonella. Green (1932) working in Malaya saw *P. inui* in a pig-tailed macaque, *M. nemestrina*.

Sinton and Mulligan (1933) discussed the '*P. inui* group' of monkey parasites and the following year Sinton (1934) redescribed the parasite in a paper which was, as Eyles wrote in 1963, "so meticulously prepared that it should serve as a model for those of us continuing to work on monkey malaria."

It appears that after the early thirties natural infections with *P. inui* were seen only rarely (Singh *et al*, 1951; and Sezen, 1956) until the Eyles group went to Malaya in 1960. Their studies and those of other investigators will be dealt with later.



S. H. Nicholson

PLASMODIUM INUI

Cycle in the Blood

PLATE XXXIX

The earliest ring forms are 2 to 3 μ in diameter, usually with a single, fairly large, nucleus. Less commonly, the nucleus may be double. As the trophozoite grows, the rings increase in size exhibiting a large vacuole and limited cytoplasm (Fig. 4). As growth continues, the parasite becomes amoeboid, stippling appears, and some pigment is evident (Figs. 6-8). Band forms, reminiscent of *P. malariae*, are not uncommon (Fig. 9). The host cell may become somewhat enlarged. As growth proceeds, the trophozoite becomes large, occupying about half the host cell, the nucleus may be pleomorphic, the pigment becomes more prominent, but is still delicate, and the large vacuole disappears. Stippling, much like Ziemann's stippling in *P. malariae*, is evident and with some Giemsa's, prominent (Figs. 13-15).

Young schizonts appear after 48 hours, and by the time 4 to 8 nuclei are evident, the parasite may almost fill the erythrocyte, or it may appear contracted with an irregular periphery. In the early stages, the nuclei may appear as if on the surface and in the company of a vacuole. Their staining progresses from reddish-black to purplish-black. Sometimes a red-staining mass (Fig. 20) is evident which disappears before the mature schizont is formed. The pigment has a greenish cast, stippling is evident but diminishes as development proceeds (Figs. 16-20).

The mature schizonts may be asymmetrical or fill the host cell. The latter, carrying the fully mature schizont, appears depleted. The pigment is coalesced and appears as a yellowish-black mass. The merozoites stain purple and number up to 18; 12 is the usual number (Figs. 21-23).

The young gametocytes are compact, have little, if any, amoeboidity, their pigment is more

abundant than in the asexual forms, and they are without a vacuole.

The cytoplasm of the adult macrogametocyte stains a delicate steel blue with scattered pigment, some of which appears to be embedded. The nucleus is eccentric and appears light red with a darker stained irregular area and a small vacuole. The parasite fills the host cell which is slightly enlarged (Fig. 24). The microgametocyte is distinctive for the way it takes the stain. The cytoplasm is reddish-purple overlaid with scattered brown pigment granules. The off-center nucleus may occupy a third or more of the parasite. The prominent reddish-purple area shows a net of thin threads which surrounds a deeper staining bar-shaped area. The parasite fills the host cell (Fig. 25).

The asexual cycle in the blood occupies 72 hours.

Sporogonic Cycle

PLATE XL

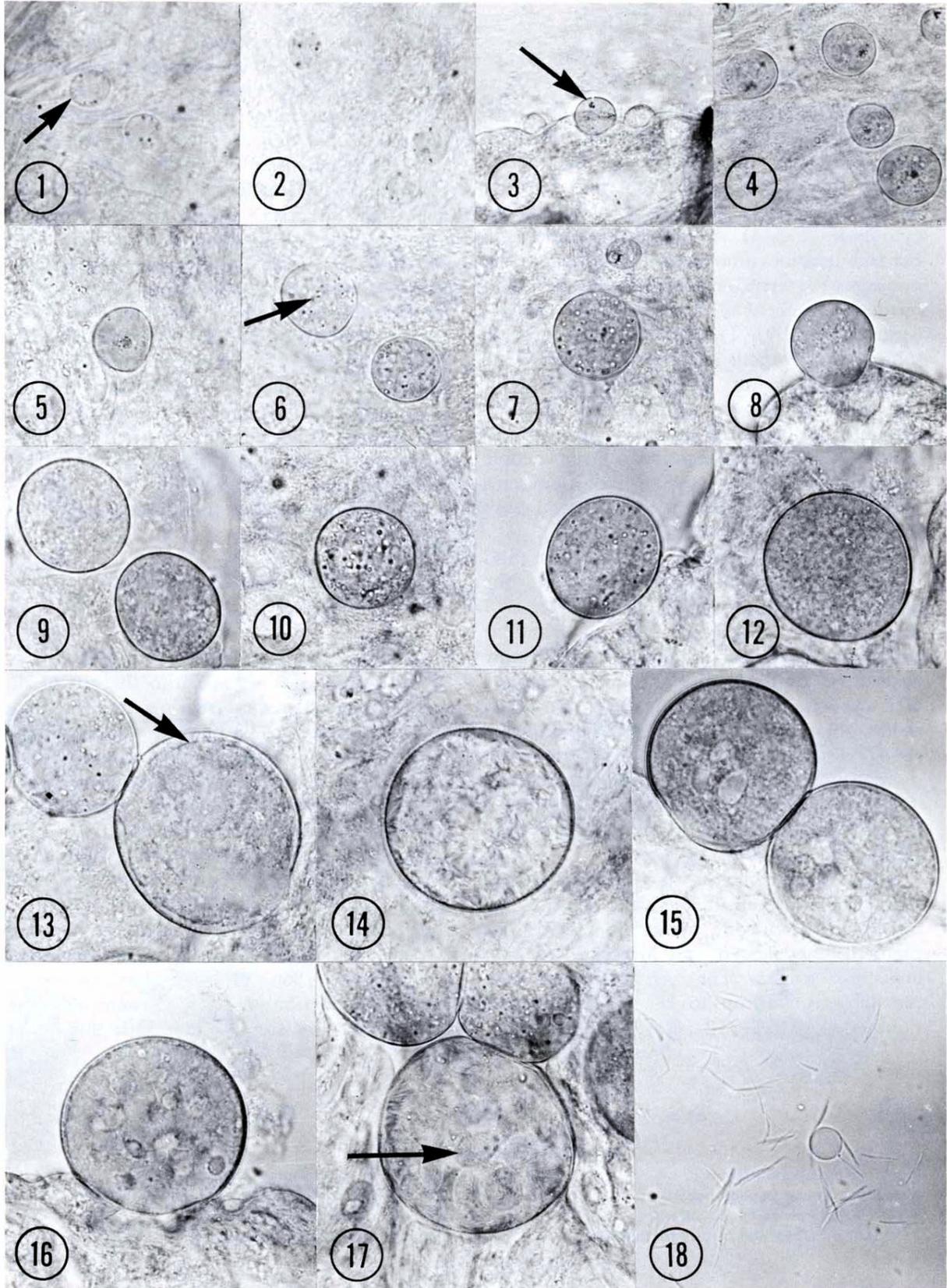
According to Bano (1959), the penetration of the ookinetes of *P. inui* into the gut of *A. aztecus* mosquitoes was very slow at an incubation temperature of 28° C. The earliest recognizable stage in many 70-hour oocysts, measuring 10 μ in diameter, was a distinct uninucleate condition suggesting a resting stage. Binucleate oocysts were observed at 72 to 79 hours; their size varied from 10.5 to 11 μ in diameter. At 81 to 83 hours, the oocysts measured 12 to 12.6 μ . A multinucleate condition was observed in the 96 to 106-hour oocysts which varied from 13.3 to 15.5 μ in diameter.

Garnham (1966) reported that oocysts in mosquitoes incubated at 25° C. were only 8 μ in diameter on the 5th and 6th day after feeding.

PLATE XXXIX.—*Plasmodium inui*.

Fig. 1. Normal erythrocyte.
Figs. 2-4. Young trophozoites.
Figs. 5-10. Growing trophozoites.
Figs. 11-15. Older and mature trophozoites.

Figs. 16-19. Developing schizonts.
Figs. 20-23. Almost mature and mature schizonts.
Fig. 24. Mature macrogametocyte.
Fig. 25. Mature microgametocyte.



On the 7th day, the oocysts measured 13 μ and the pigment was concentrated in crossed lines at the center. On the 14th day, the oocysts measured 22 μ but maturity was not attained until the 21st day. At 28° C, the oocysts grew more quickly, and after 12 days, reached 40 μ and after 14 days, a size of 50 μ. Just before the final differentiation, Garnham noted a number of darkly staining spheres, 7 μ in diameter. These seemed to give rise to vesicles, 10 μ in diameter, in which sporozoites were discerned. Malaria pigment was usually visible throughout the growth of the oocyst.

In our studies (Table 32), the oocyst growth rate was observed in four species of mosquitoes incubated at 25° C. In *A. b. balabacensis*, after 6 days of extrinsic incubation, the oocyst diameters ranged from 9 to 17 μ with a mean of 13 μ. The oocysts grew slowly, so that by day 14, the mean size was 50 μ with a range of 15 to 73 μ. At this time, differentiation within the oocyst was apparent. On the 15th day, sporozoites were present in the salivary glands.

In *A. freeborni* and *A. maculatus* mosquitoes, the mean oocyst diameters were noticeably smaller than in the *A. b. balabacensis*. This was probably due to the

TABLE 32.—Oocyst diameters of *Plasmodium inui* in *Anopheles b. balabacensis*, *A. freeborni*, *A. maculatus*, and *A. quadrimaculatus*.

Days after Infection	<i>A. b. balabacensis</i>			<i>A. freeborni</i>			<i>A. maculatus</i>			<i>A. quadrimaculatus</i>		
	No.	Range*	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
6	100	9-17	13	114	7-14	10	23	9-13	12			
7	131	8-21	15	216	8-18	13	267	8-18	13	211	8-20	14
8	150	12-27	21	308	9-27	16	293	8-20	15	215	8-21	16
9	100	15-34	27	164	8-30	15	315	9-30	19	203	12-30	21
10	100	21-38	32	163	9-28	17	193	9-30	19	200	12-32	22
11	148	15-51	36	229	11-40	24	207	11-45	21	123	12-45	27
12	150	17-44	31	245	9-50	29	199	9-50	25	223	12-55	37
13	50	22-50	38	175	9-68	30	226	11-55	26†	244	14-71	40†
14	150	15-73	50†	132	11-68	42†	366	12-63	35†	291	15-68	48†
15	50	22-53	37†**	88	11-53	37†	359	13-78	41†	225	21-77	52†
16							132	13-65	34†**	130	15-79	49†
Totals	1129	8-73		1836	7-68		2580	8-78		2065	8-79	

* Measurements expressed in microns; incubation temperature 25° C

† Oocyst differentiation.

** Sporozoites present in the salivary glands.

Plate XL.—Developing oocysts and sporozoites of *Plasmodium inui* (Mulligan strain) in *Anopheles b. balabacensis* mosquitoes. X 580 (Except Figures 1 and 2).

- Fig. 1. 6-day oocysts showing a few pigment granules near periphery. X 740.
- Fig. 2. 7-day oocysts. X 740.
- Fig. 3. 8-day oocyst showing clumping of pigment.
- Fig. 4. 9-day oocysts showing clumped pigment.
- Fig. 5. 10-day oocyst.
- Fig. 6. 11-day oocysts showing pigment and small vacuoles.
- Fig. 7. 12-day oocysts showing one of normal size and one much smaller.
- Fig. 8. 12-day oocyst.
- Fig. 9. 13-day oocysts.
- Fig. 10. 14-day oocyst.
- Fig. 11. 15-day oocyst.
- Fig. 12. 15-day oocyst.
- Fig. 13. 16-day oocyst showing early signs of differentiation.
- Fig. 14. 16-day differentiating oocyst.
- Fig. 15. 17-day oocysts.
- Fig. 16. 17-day oocyst.
- Fig. 17. 17-day oocyst showing sporoblast formation.
- Fig. 18. Sporozoites emerging from salivary gland tissue 17 days after feeding.

presence of a large number of small oocysts in each of these two species. Oocyst differentiation was apparent in the *A. freeborni* mosquitoes by day 14. However, sporozoites did not appear in the salivary glands until 18 or 19 days after feeding, and then, at a low level. In *A. maculatus*, oocyst differentiation was apparent by day 13 and sporozoites were present in the salivary glands after 16 days of extrinsic incubation. Fewer of the *A. maculatus* (99 out of 313) had infected salivary glands than did the *A. b. balabacensis* (95 out of 144) after 15 days of incubation. The intensity of gland infections was high in both species.

In *A. quadrimaculatus*, the oocyst growth rate was similar to that in *A. b. balabacensis*. Differentiation was apparent after 13 days, but no positive salivary glands were found until day 20, and then, at a very low level.

A comparison of the oocyst growth rate of *P. inui* with that of *P. cynomolgi* in *A. b. balabacensis* mosquitoes (Fig. 54) shows that the *P. inui* are smaller and that sporozoites appear in the salivary glands 5 days later than in *P. cynomolgi*.

Garnham (1966) reported that sporozoites may be found in the salivary glands 15 to 17 days after feeding when the mosquitoes are incubated at 28° C. At a temperature of 25° C, the development requires 3 weeks. His experimental mosquito hosts were not ideal (*A. aztecus* and *A. atroparvus*) and sporozoites disappeared or became scarce in the salivary glands in a few days. In dried preparations, the sporozoites measured 12 to 13 μ in length.

Transmission of *P. inui* was first obtained by Garnham (1951) through the bites of infected *A. atroparvus* mosquitoes coupled with the intravenous inoculation of infected salivary glands. Mohiuddin (1957) passed the parasite to *M. mulatta* by the inoculation of dissected glands from infected *A. aztecus*. He also allowed infected *A. atroparvus* and *A. aztecus* mosquitoes to feed on a monkey which later developed a patent infection. It is impossible, in this instance, to say which of the species transmitted the infection, maybe both. Eyles (1960) obtained infections by the intravenous inoculation of salivary glands from infected *A. quadrimaculatus*. Shortt *et al* (1963) transferred

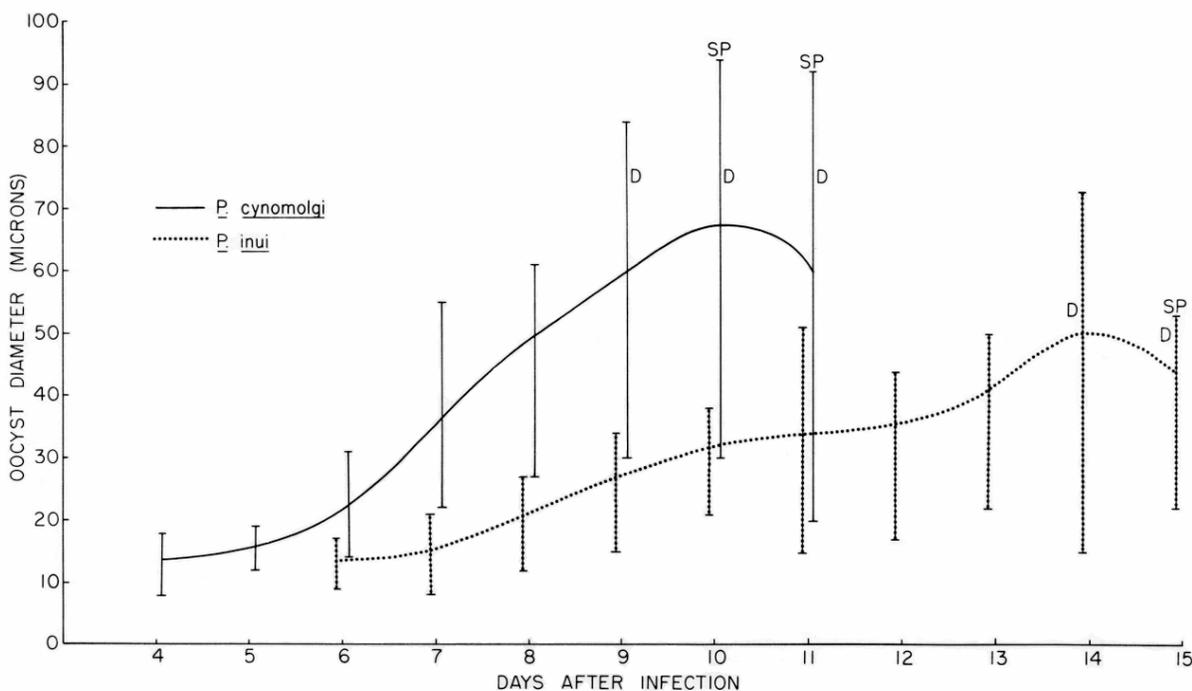


FIGURE 54.—Median oocyst diameter curves and ranges in oocyst diameters of *Plasmodium cynomolgi* and *Plasmodium inui* in *Anopheles b. balabacensis* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

the parasite by the intravenous inoculation of salivary glands containing sporozoites from *A. aztecus* and from *A. stephensi*.

In our studies (Collins *et al*, 1966, 1968), we obtained transmission of 12 different isolates of *P. inui* by the bites of *A. b. balabacensis*, *A. maculatus*, or *A. stephensi* mosquitoes. Among a total of 18 transmissions, the prepatent periods ranged from 10 to 26 days with a mean of 16.4 days. Twelve additional passages have been obtained by the intravenous and/or intrahepatic inoculation of sporozoites from *A. freeborni* (twice), *A. quadrimaculatus* (twice), and *A. maculatus* mosquitoes (8 times). The prepatent periods ranged from 11 to 31 days with a mean of 17 days.

Transmission of the infection to man (Coatney *et al*, 1966) was made via the bites of *A. maculatus* and *A. stephensi* mosquitoes. The prepatent periods in the volunteers were 31 and 56 days.

Cycle in the Tissue

PLATE XLI

Garnham (1951) was the first to see EE stages of *P. inui* which he described and presented in a series of 15 figures. In this work, he gave a single rhesus monkey sporozoites from *Anopheles m. atroparvus* mosquitoes infected with the Mulligan strain on 2 consecutive days. Liver biopsies were taken on days 8 and 12, after the first exposure to infection, and in that material he found EE stages of variable size. Garnham assumed that the smaller forms in each of the biopsies, resulted from sporozoites injected on the second day and the larger forms from those of the first day. On that basis, he described 7-, 8-, 11-, and 12-day forms. It is not impossible that the same variation in size might have resulted had the animal been exposed on only a single day. In 1966, Garnham described 9- and 10-day forms of the parasite but gave no details regarding the strain of *P. inui* employed or the method used in obtaining the material for the descriptions.

Shortt *et al* (1963) described 8- and 9-day EE forms of *P. osmaniae*, later named *P. shortti* Bray, 1963; we consider the parasite to be *P. inui* for reasons explained later.

Held *et al* (1968) were well aware of the uncertainty regarding the taxonomy of certain quartan parasites of simians and elected to study the exoerythrocytic stages of the parasite in 4 different strains: 1) The L strain isolated from an *A. leucosphyrus* mosquito by our unit in Kuala Lumpur, Malaysia. Its blood stages are indistinguishable from the original strain isolated by Sinton; 2) The OS strain, employed by Coatney *et al* (1966) in their successful attempt to infect man. The origin and the designation of the OS strain are described in the section dealing with Course of Infection; 3) The CDC strain, isolated from a long-tailed macaque, *M. fascicularis*, received directly from the Philippines, and indistinguishable from the original Sinton-isolated strain; 4) The Philippine strain, isolated by Lambrecht *et al* (1961) and designated the Cebu strain.

Infection of clean rhesus monkeys was by intrahepatic inoculation of sporozoites and the site of the inoculation was marked by a stainless steel ligature. To make sure of the age of the EE bodies found subsequently, each animal received infective material on a single day. EE bodies of the L strain were found in 6-, 7-, 8-, and 9-day biopsy material; in OS strain material at 6, 7, & 8 days; in the CDC strain material, at 7 days; and in Philippine strain material at 10 days, following exposure.

In each of the four strains, the EE, bodies were round or slightly elliptical. The edges were smooth. The nuclei stained magenta, were generally round, although other shapes were seen, and measured 0.5 to 1.0 μ in diameter. The cytoplasm stained a pale blue. Dark blue cytoplasmic aggregates up to 6 μ in diameter were seen in some older forms. Some EE bodies took a deeper stain than others giving the impression of compactness. Small pink staining vacuoles, less than 3 μ in diameter, were seen in a few sections. Some sections exhibited a separation of 1 to 5 μ between the parasite and the host tissue. Each of the EE bodies was measured and the results compared with the measurements given by Garnham (1951, 1966) and by Shortt *et al* (1963).

The EE bodies studied by Held *et al* were considerably larger than corresponding ones described by Garnham and by Shortt *et al*. The latter authors (1963) in their description of the

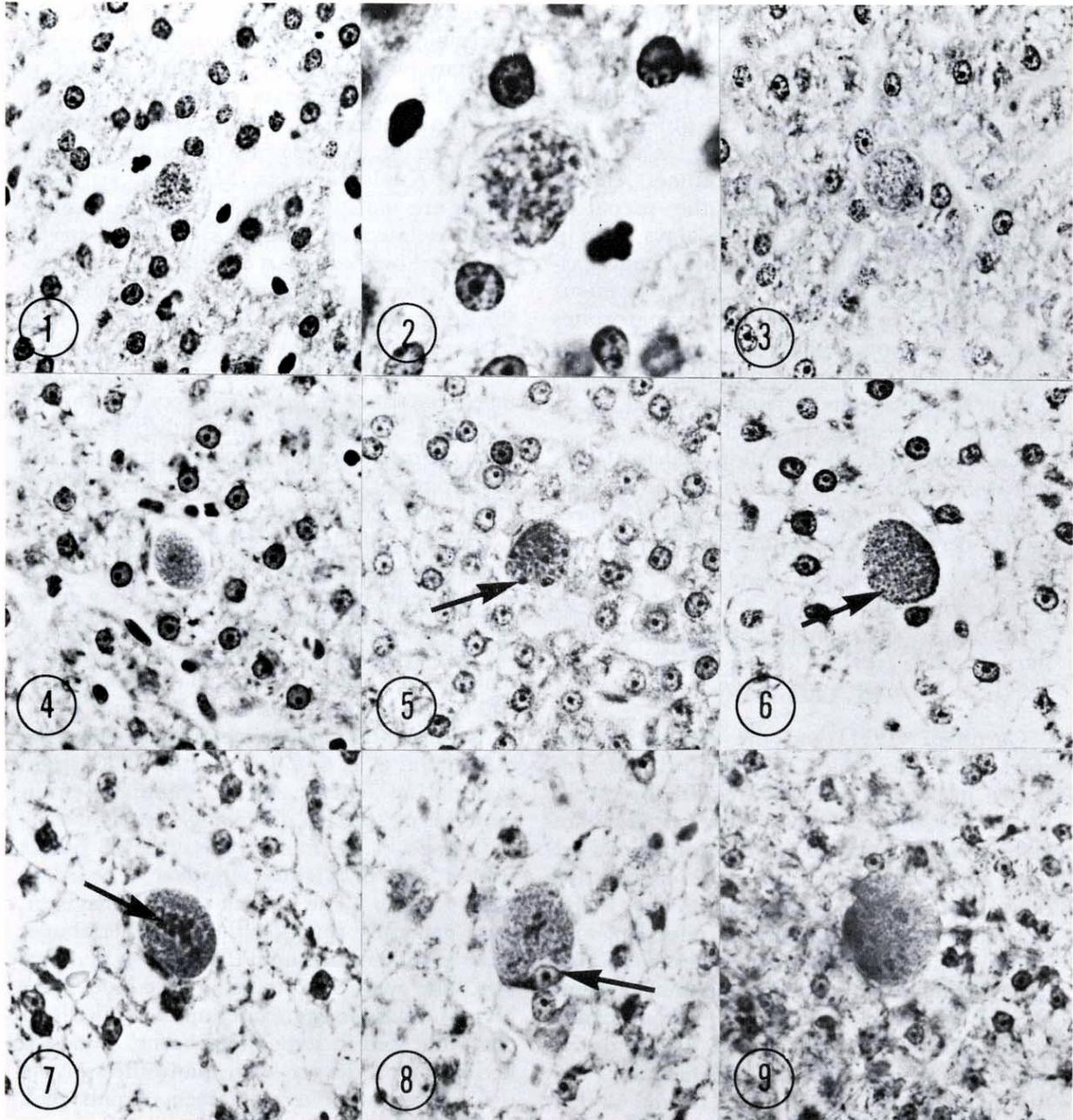


PLATE XLI—Exoerythrocytic bodies of *Plasmodium inui* in liver tissue of *Macaca mulatta*. X 580 (Except Figs. 2 and 8). Figures

- 1, 2, 4, 6, 7, and 8 are examples of the Leucophyrus strain; Figures 3 and 5 of the OS strain; Figure 9 of the Philippine strain.
- Fig. 1. 6-day body.
 Fig. 2. 6-day body. X 1450.
 Fig. 3. 7-day body.
 Fig. 4. 7-day body.
 Fig. 5. 8-day body showing prominent flocculi.
 Fig. 6. 8-day body showing individual nuclei.
 Fig. 7. 9-day body showing clumped flocculi.
 Fig. 8. 9-day body showing flocculi and host cell nucleus. X 742.
 Fig. 9. 10-day body.

EE bodies of *P. osmaniae* stressed their size, they were larger than *P. inui*, as an argument for species status and Garnham (1967) supported that view. If that criterion were accepted, then each of the strains studied by the Held group would represent separate species. We hold that this is untenable, for the present at least, because all other essential characters of these strains are so similar. And, too, observations on a few EE bodies which lack consistent specific morphologic differences, with the possible exception of size at a given time after infection, can hardly supplant the results of prolonged study of the blood stages by competent investigators over many years.

Course of Infection

Although *P. inui* infects many species of monkeys, most of the studies have been confined to the rhesus monkey, *M. mulatta*. Eyles (1963) stated that infections produced in this animal were moderate when compared to *P. cynomolgi* and remarkably long lasting. In our own experience, many animals have maintained infections for many years.

A perusal of the median parasitemia curve for 66 intact *M. mulatta* monkeys infected with *P. inui* by the inoculation of parasitized blood

will show (Fig. 55) a peak parasitemia of approximately 35,000 per mm^3 on the 14th day of patent parasitemia. The parasitemia then dropped and fluctuated between 4,000 and 6,000 per mm^3 for the remainder of the 60-day observation period. Such levels were commonly maintained for months and even years. In some individual monkeys, parasite levels of 100,000 per mm^3 or greater were maintained for many months. Sporozoite induced infections (20 monkeys) rose to a peak of approximately 15,000 per mm^3 by day 14 and thereafter dropped to a level of approximately 2,500 per mm^3 after 60 days. In splenectomized monkeys (27 animals), the peak parasitemia of approximately 500,000 per mm^3 was obtained after 16 days. This dropped rapidly to a level of 70,000 per mm^3 after 30 days of patent parasitemia. Some of the animals continued to maintain a high level of parasitemia for many months. Garnham (1966) reports that *M. mulatta* monkeys which have severe infections are apt to develop a sterilizing immunity and throw off the infection entirely. He also reported a similar occurrence of splenectomized monkeys. We have not had this experience. Some of our animals have maintained fairly high parasitemias for 3 to 4 years after splenectomy.

There is no evidence that *P. inui* possesses

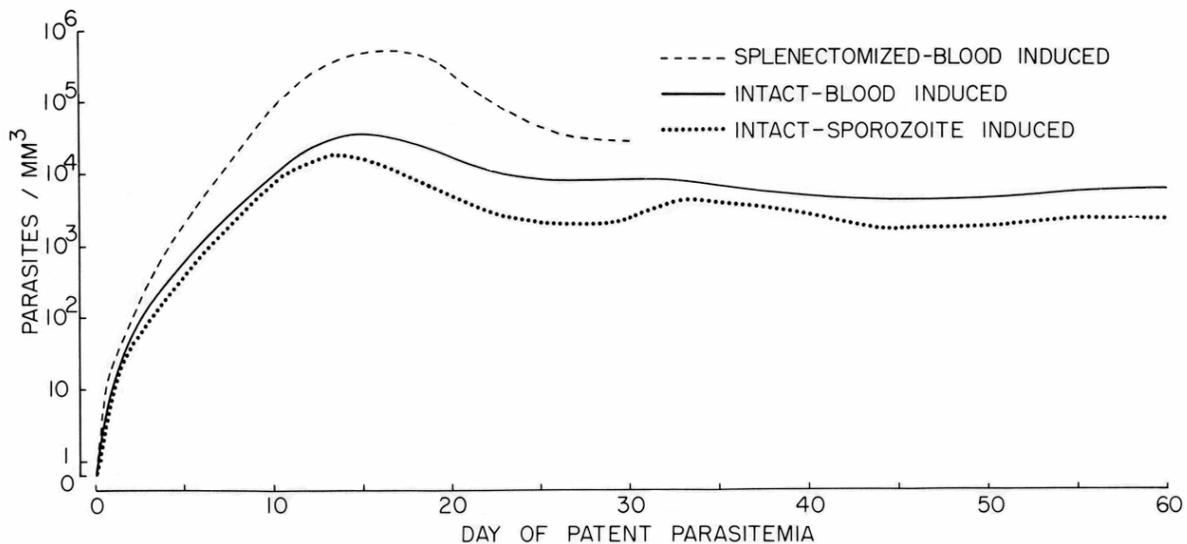


FIGURE 55.—Median parasitemia curves for 113 infections of *Plasmodium inui* in *Macaca mulatta* monkeys (66 blood-induced and 20 sporozoite-induced infections in intact animals, and 27 blood-induced infections in splenectomized animals).

the mechanism for true relapse. Several of our monkeys, infected by sporozoite inoculation, had their blood infection cured by the administration of schizontocidal drugs. They failed to develop secondary infections during a 6-month observation period. Later, they were shown to be susceptible to reinfection when inoculated with parasitized blood of the same strain.

Infections in the *M. fascicularis* and *M. radiata* monkeys are usually less severe than those in the rhesus monkey (Garnham, 1966).

Plasmodium inui is infectious to man as shown by Das Gupta (1938). The volunteer was given blood directly from an infected *S. irus* (= *M. fascicularis*) monkey by intramuscular injection. Twenty-three days later the patient developed a patent parasitemia and febrile symptoms on the 28th day, which continued for a total of 3 days. The maximum temperature observed was 102° F. The strain of *P. inui* employed by Das Gupta was obtained directly from Col. Sinton which leaves little doubt as to the identity of the parasite.

Our own studies in man (Coatney *et al.*, 1966) were carried out with the OS strain of *P. inui*. The name originally suggested for the parasite (Shortt *et al.*, 1961) was *P. osmaniae*. Because that name was only a suggested one, Bray (1963) proposed the name *P. shortti*. Eyles obtained the parasite through the courtesy of Professor Garnham and after carefully comparing it with the original strain of *P. inui* was unable, on morphological grounds, to separate it from the original strain. Nevertheless, he elected to consider it a subspecies, *P. inui shortti*. The parasite was sent to us from our installation in Kuala Lumpur, Malaysia, and after careful study, we agreed with Eyles that it was an inui-type parasite; until further studies convince us of its taxonomic status, we prefer to designate the osmaniae-shortti parasite as the OS strain of *P. inui*.

Two Caucasian male volunteers were exposed to the bites of infected mosquitoes and 5 other volunteers, including one Negro, received their infections by the intravenous inoculation of parasitized blood from one of the previously infected volunteers.

One volunteer was bitten by 5 *Anopheles maculatus* and by 7 *A. stephensi*. The other,

received bites from 19 *A. maculatus*. Infection of the mosquitoes was proved by postprandial dissection. Each man became infected. The prepatent periods were 31 and 56 days; and parasitemia continued for 21 days in one and for 24 days in the other. The maximum parasite count was 2,520 per mm³ of blood. Each of the 5 volunteers who received parasitized blood developed infections which were patent for 10 to 26 days with a maximum parasite count of 450 per mm³ (Fig. 56).

The quartan pattern was well marked in two of the volunteers with maximum fever of 103.2° F. The others exhibited low-grade, remittent-type fever or, in the case of the Negro patient, no fever at all. No morphological changes were observed in the parasites as a result of their sojourn in man. Blood from 4 of the volunteers was inoculated into clean rhesus monkeys which produced typical *P. inui* infections.

The major complaints voiced by the volunteers were headache, malaise, muscular and joint pains, and loss of appetite. When chills occurred, they were mild and of short duration. In no case was anti-malarial therapy necessary.

Some interesting biological facts emerged as a result of this study. Blood was drawn from one volunteer on day 16, following his exposure to infection by mosquito bite, and given to another, who developed a patent infection 47 days later. At the time blood was drawn, the donor had mild symptomatic complaints although parasites could not be demonstrated in his blood until day 56. The fact that the recipient became infected shows that tissue schizogony had taken place by day 16 and, further, that only a small number of parasites is needed to establish a patent infection in man.

It is well known that Negroes are universally susceptible to *P. malariae*, the human quartan parasite, and we had shown that the quartan parasite of New World monkeys, *P. brasilianum* (see Chapter 19) and *P. inui* of Old World monkeys are infective to Negroes. In contrast to these successes, we have not been able to infect Negroes with tertian parasites, *P. cynomolgi* (see Chapter 6) and *P. schwetzi* (see Chapter 12). Likewise, it is sometimes difficult to infect Negroes with the human tertian parasite, *P. vivax* (see Chapter 5). So far we cannot account for this resistance phenomenon.

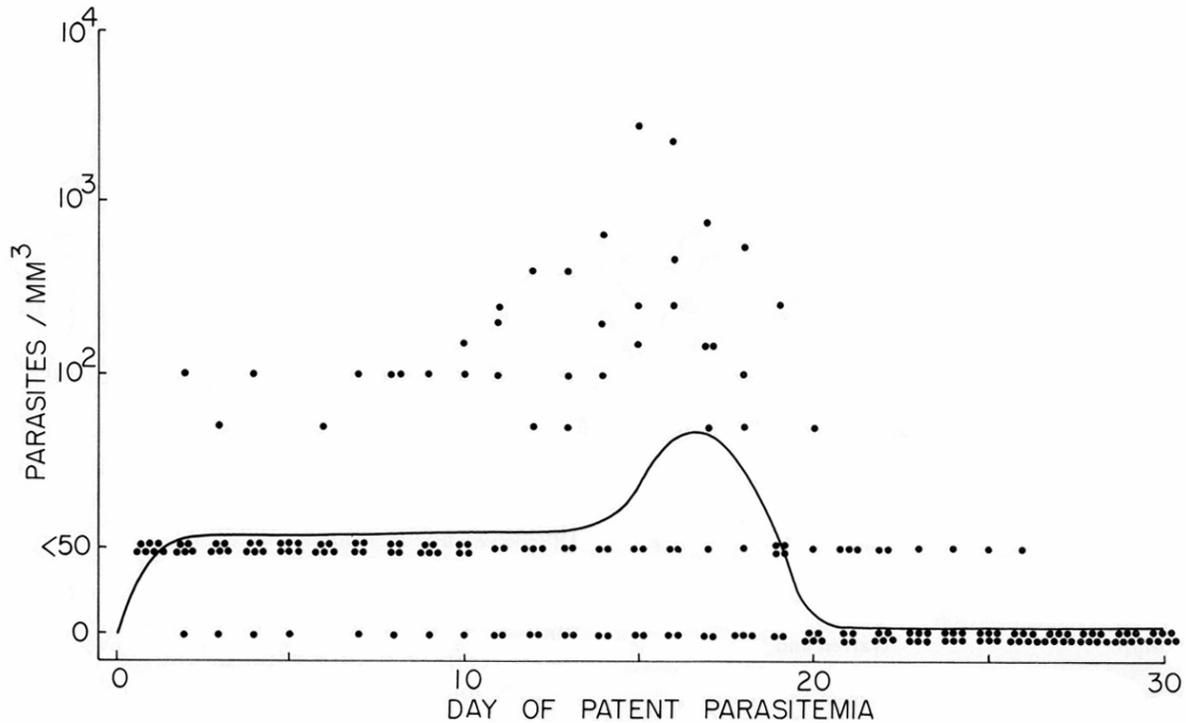


FIGURE 56.—Parasitemia and median parasitemia curve for 7 infections of *Plasmodium inui* (OS strain) in human volunteers.

It probably should be mentioned, too, that prior to, and subsequent to, our successful transmission of this strain of *P. inui* to man, by mosquito bite, we had made several attempts, using other strains, each time without success. To some this might indicate that true *P. inui* would not grow in man. Since the osmaniae-shortti parasite did infect man, some might consider that reason enough for accepting it as an entity outside the inui group. However, as mentioned earlier, Das Gupta was able to infect man with the original strain of *P. inui* following inoculation of parasitized blood. Why our earlier attempts failed and this one succeeded, we are not prepared to say.

Host Specificity

Plasmodium inui naturally infects many monkeys as listed below:

SPECIES	REFERENCES
<i>Cercopithecus mitis</i> (Experimentally)	Garnham, 1966
<i>Cynopithecus niger</i>	Eyles and Warren, 1962
<i>Macaca cyclopis</i>	Hsieh, 1960
<i>M. fascicularis</i>	Many references
<i>M. mulatta</i>	Schmidt <i>et al</i> , 1961; Notananda <i>et al</i> , 1961
<i>M. nemestrina</i>	Halberstaedter and von Prowazek, 1907
<i>M. radiate</i>	Mulligan and Swaminath, 1940
<i>Presbytis cristatus</i>	Collins <i>et al</i> , 1970
<i>P. obscurus</i>	Eyles <i>et al</i> , 1962

Plasmodium inui has been isolated from *Anopheles leucosphyrus* (Wharton *et al*, 1962) and *A. b. introlatus* (Warren and Wharton, 1963), in Malaya. Experimentally, *P. inui* is infectious to a large number of mosquitoes as listed below:

SPECIES	REFERENCES
<i>Anopheles albimanus</i>	Eyles, 1960a
<i>A. atroparvus</i>	Weyer, 1937; Garnham, 1951; Mohiuddin, 1957; Shortt <i>et al</i> , 1963
<i>A. aztecus</i>	Mohiuddin, 1957; Bano, 1959; Shortt <i>et al</i> , 1963; Garnham, 1966
<i>A. b. balabacensis</i>	Collins <i>et al</i> , 1968
<i>A. freeborni</i>	Eyles, 1960
<i>A. gambiae</i>	Garnham, 1951
<i>A. letifer</i>	Warren and Wharton, 1963
<i>A. maculatus</i>	Warren and Wharton, 1963; Collins <i>et al</i> , 1966, 1968
<i>A. philippinensis</i>	Warren and Wharton, 1963
<i>A. quadrimaculatus</i>	Eyles, 1960
<i>A. stephensi</i>	Garnham, 1951; Shortt <i>et al</i> , 1963; Collins <i>et al</i> , 1966, 1968

In addition to the above, we have obtained infections in *A. kochi*, *A. vagus*, *A. sinensis*, and *A. riparis*. Thirteen species of mosquitoes are listed (Table 33) for comparison as to their susceptibility to infection with *P. inui*. The most readily infected was *A. b. balabacensis* and the least was *A. letifer*.

Immunity and Antigenic Relationships

Singh and Singh (1940) observed that homologous superinfection with *P. inui* resulted in a very mild and transient parasitemia. Chronic infections with *P. inui* afforded no protection against either *P. cynomolgi* or *P. knowlesi*. Monkeys with chronic infections of *P. cynomolgi* or *P. knowlesi* showed no resistance to typical infections with *P. inui*.

In contrast, Voller *et al.* (1966) found that monkeys immune to infection with *P. knowlesi*

or *P. shortti* (= *P. inui*) developed low parasitemias of *P. inui* upon challenge. Voller and Rossan (1969) confirmed this observation by finding that in monkeys with chronic infections of *P. knowlesi*, infections with *P. inui* developed more slowly and had lower peak parasitemias than did animals infected with *P. inui* alone.

Antisera to *P. inui* gave a fluorescent antibody cross-reaction at a very high level to *P. fieldi* and *P. shortti* (= OS strain *P. inui*) (mean reciprocal titer ratios of 100:107 and 100:88) and reacted at somewhat lower levels to *P. brasilianum*, *P. coatneyi*, and *P. cynomolgi* (mean reciprocal titer ratios of 100:57, 100:57, and 100:46, respectively). In the reverse procedure, *P. inui* antigen cross-reacted to none of the heterologous antisera at a high level, the highest response was to the OS strain with a mean reciprocal titer ratio of 100:35 (Collins *et al*, 1966). Antigen to the OS strain gave fluorescent antibody cross-reactions of a much lower magnitude with *P. fieldi* and *P. inui* giving the highest levels of response (mean reciprocal titer ratios of 100:47 and 100:35, respectively). In the reverse procedure, the OS strain antigen gave a high level cross-reaction to the *P. inui* antigen only (mean reciprocal titer ratio of 100:88).

In a further study of antigenic relationships (Collins *et al*, 1970), antisera from monkeys each infected with one of 15 different isolates of *P. inui* from different geographical and ecological areas of South Central and Southeast Asia were allowed to react with their homologous and heterologous antigens using the fluorescent antibody technique. It was shown, using the test of relatedness statistic, that there were significant antigenic differences between each pair of isolates of *P. inui* included in the study. The OS strain parasite was, on the average, more distantly related to the remainder of the strains than was any other strain. The next most distantly related was a parasite from the Celebes.

El-Nahal (1967) reported that antisera to *P. inui* and *P. shortti* (= OS strain *P. inui*) failed to respond to exoerythrocytic antigens of *P. cynomolgi* and *P. malariae* using a fluorescent antibody procedure.

TABLE 33.—Comparative infectivity of *Plasmodium inui* to thirteen species of *Anopheles*.

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
Bal						100
Bal : F-1	63	492	629	83.9	65.5	59.9
Bal : Kochi	5	131	88	93.9	87.5	52.1
Bal : St-1	109	991	759	66.1	69.2	44.9
Bal : Mac	23	123	208	80.5	68.3	41.4
Bal : Q-1	83	1223	1176	47.9	38.6	17.8
Bal : Vagus	6	74	29	83.8	62.1	9.6
Bal : Sin	2	31	17	83.9	17.6	9.2
Bal : Atro	29	583	570	34.6	15.3	7.1
Bal : Phil	1	16	2	87.5	100	4.2
Bal : Rip	2	61	3	88.5	100	3.8
Bal : Alb	46	696	835	39.4	5.1	1.5
Bal : Let	5	66	50	80.3	10.0	1.3

* Bal = *Anopheles b. balabacensis*; F-1 = *A. freeborni*; Kochi = *A. kochi*; St-1 = *A. stephensi*; Mac = *A. maculatus*; Q-1 = *A. quadrimaculatus*; Vagus = *A. vagus*; Sin = *A. sinensis*; Atro = *A. atroparvus*; Phil = *A. philippinensis*; Rip = *A. riparis*; Alb = *A. albimanus*; Let = *A. letifer*.

** 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.

REFERENCES

- BANO, L., 1959. A cytological study of the early oocysts of seven species of *Plasmodium* and the occurrence of postzygotic meiosis. *Parasitology* 49 : 559-585.
- BOUILLIEZ, M., 1913. Nouvelles recherches experimentales sur un *Plasmodium* des singes. *C. R. Soc. Biol. (Paris)* 74: 1070-1072.
- BRAY, R. S., 1963. Malaria infections in primates and their importance to man. *Ergeb. Mikrob. Immunit. Experim. Therap.* 36 : 168-213.
- COATNEY, G. R., CHIN, W., CONTACOS, P. G., and KING, H. K., 1966. *Plasmodium inui*, a quartan-type malaria parasite of Old World monkeys transmissible to man. *J. Parasit.* 52 : 660-663.
- COLLINS, W. E., CONTACOS, P. G., GUINN, E. G., and HELD, J. R., 1966. Studies on the transmission of simian malaras. I. Transmission of two strains of *Plasmodium inui* by *Anopheles maculatus* and *A. stephensi*. *J. Parasit.* 52 : 664-668.
- COLLINS, W. E., CONTACOS, P. G., GUINN, E. G., and HELD, J. R., 1968. Some observations on the transmission of *Plasmodium inui*. *J. Parasit.* 54 : 846-847.
- COLLINS, W. E., SKINNER, J. C., and GUINN, E. G., 1966. Antigenic variations in the plasmodia of certain primates as detected by immuno-fluorescence. *Am. J. Trop. Med. & Hyg.* 15 : 483-485.
- COLLINS, W. E., WARREN, McW., SKINNER, J. C., and ALLING, D. W., 1970. *Plasmodium inui*: Serologic relationships of Asian isolates. *Exp. Parasit.* 27 : 507-515.
- DAS GUPTA, B. M., 1938. Transmission of *P. inui* to man. *Proc. Natl. Inst. Sci. India* 4: 241-244.
- EL-NAHAL, H. M. S., 1967. Study of serological cross-reactions of exo-erythrocytic schizonts of avian, rodent and primate malaria parasites by the fluorescent antibody technique. *Bull. Wid. Hlth. Org.* 37 : 154-158.
- EYLES, D. E., 1960. *Anopheles freeborni* and *A. quadrimaculatus* as experimental vectors of *Plasmodium cynomolgi* and *P. inui*. *J. Parasit.* 46 : 540.
- EYLES, D. E., 1960a. Susceptibility of *Anopheles albimanus* to primate and avian malaras. *Mosq. News* 20 : 368-371.
- EYLES, D. E., LAING, A. B. G., WARREN, McW. and SANDOSHAM, A. A., 1962. Malaria parasites of Malayan leaf monkeys of the genus *Presbytis*. *Med. J. Malaya* 17 : 85-86.
- EYLES, D. E. and WARREN, McW., 1962. *Plasmodium inui* in Sulawesi. *J. Parasit.* 48: 739.
- EYLES, D. E., 1963. The species of simian malaria: taxonomy, morphology, life cycle, and geographical distribution of the monkey species. *J. Parasit.* 49 : 866-887.
- GARNHAM, P. C. C., 1951. The mosquito transmission of *Plasmodium inui* Halberstaedter and von Prowazek, and its pre-erythrocytic development in the liver of the rhesus monkey. *Trans. Roy. Soc. Trop. Med. & Hyg.* 45 : 45-52.
- GARNHAM, P. C. C., 1966. Malaria parasites and other haemosporidia. Blackwell Scientific Publications. Oxford. pp.1114.
- GARNHAM, P. C. C., 1967. Abstract of a paper by Coatney *et al*, 1969. *Trop. Dis. Bull.* 64 : 464.
- GREEN, R., 1932. A malarial parasite of Malayan monkeys and its development in anopheline mosquitoes. *Trans. Roy. Soc. Trop. Med. & Hyg.* 25 : 455-477.
- HALBERSTAEDTER, L. and VON PROWAZEK, S., 1907. Untersuchung über die malariparasiten der affen. *Arb. K. Gesundh.-Amte (Berl.)* 26 : 37-43.
- HELD, J. R., CONTACOS, P. G., JUMPER, J. R., and SMITH, C. S., 1968. Studies of the exoerythrocytic stages of simian malaria. III. *Plasmodium inui*. *J. Parasit.* 54 : 249-254.
- HSIEH, H. C., 1960. Malaria parasites of the Tiawan monkey. *Formosan Science.* 14: 477-487.
- LAMBRECHT, F. L., DUNN, F. L., and EYLES, D. E., 1961. Isolation of *Plasmodium knowlesi* from Philippine macaques. *Nature.* 191 : 1117-1118.
- LEGER, M. and BOUILLIEZ, M., 1912. Sur un *Plasmodium* des singes. Passages par especes variees. *Action pathogene.* *C. R. Soc. Biol.* 73 : 310-313.
- LEGER, M. and BOUILLIEZ, M., 1913. Recherches experimentales sur "*Plasmodium inui*" Halberstadter et

REFERENCES—Continued

- Prowazek d'un "Macacus cynomolgus". Ann. Inst. Pasteur. 27 : 955-985.
- MATHIS, C. and LEGER, M., 1911. Plasmodium des macaques du tonkin. Ann. Inst. Pasteur. 25 : 593-601.
- MAYER, M., 1907. Ueber malaria beim affen. Med. Klin. 3 : 579-580.
- MOHIUDDIN, A., 1957. Notes on a new strain of "Plasmodium inui". Riv. di. Malariol. 36 : 203-208.
- MULLIGAN, H. W. and SWAMINATH, C. S., 1940. Natural infection with *Plasmodium inui* in *Silenus sinicus* from South India. J. Malar. Inst. Ind. 3 : 603-604.
- NOGUCHI, H., 1928. Etiology of oroya fever. XII. Influence of malarial infection (*Plasmodium inui*), splenectomy, or both, upon experimental carrion's disease in monkeys. J. Exp. Med. 47 : 812-827.
- NOTANANDA, V., NILUBOL, S., and SWASDIWONGHORN, P., 1961. A preliminary note on the discovery of simian malaria in Chingmai. Minuten Nadeln. 1 : 27.
- SCHMIDT, L. H., GREENLAND, R., ROSSAN, R. and GENTHER, C., 1961. The occurrence of malaria in wild-caught rhesus monkeys. Science 133 : 753.
- SEZEN, N., 1956. Studies on the life-cycle of two strains of *Plasmodium inui* and the development of immunity. Turk. Ij. tecz. Biyol. Dreg. 16 : 240-242. (NS).
- SHORTT, H. E., RAO, G., OADRI, S. S., and ABRAHAM, R., 1961. *Plasmodium osmaniae*, a malaria parasite of an Indian monkey *Macaca radiata*. Jour. Trop. Med. & Hyg. 64 : 140-143.
- SHORTT, H. E., BAKER, J. R. and NESBITT, P. E., 1963. The pre-erythrocytic stage of *Plasmodium osmaniae*. Jour. Trop. Med. & Hyg. 66 : 127-129.
- SINGH, J. and SINGH, H., 1940. Observations on immunity in monkey malaria as evidenced by the results of superinfection. J. Malaria Inst. India 3 : 99-114.
- SINGH, J., RAY, A. P., NAIR, a. P., and BASU, P. a., 1951. Isolation of a strain of *P. inui* from mixed infection in Malayan monkey. Ind. Jour. Malariol. 5 : 433-445.
- SINTON, J. A. and MULLIGAN, H. W., 1933. A critical review of the literature relating to the identification of the malarial parasites recorded from monkeys of the families aercopithecidae and aolobidae. Rec. Mal. Surv. India III: 381-443.
- SINTON, J. A., 1934. A quartan malaria parasite of the lower oriental monkey, *Silenus irus* (*Macacus cynomolgus*). Rec. Mal. Surv. India 4 : 379-410.
- VOLLER, A., GARNHAM, P. C. C. and TARGETT, G. A. T., 1966. Cross immunity in monkey malaria. Jour. Trop. Med. & Hyg. 69 : 121-123.
- VOLLER, A. and ROSSAN, R. N., 1969. Immunological studies on simian malaria parasites. IV. Heterologous superinfection of monkeys with chronic *Plasmodium knowlesi* infections. Trans. Roy. Soc. Trop. Med. & Hyg. 63 : 837-845.
- WARREN, McW., and WHARTON, R. H., 1963. The vectors of simian malaria: identity, biology, and geographical distribution. J. Parasit. 49 : 892-904.
- WHARTON, R. H., EYLES, D. E., WARREN, McW. and MOORHOUSE, D. E., 1962. *Anopheles leucosphyrus* identified as a vector of monkey malaria in Malaya. Science 137 : 758.
- WEYER, F., 1937. Versuche zur ubertragung der affen-malaria durch stechmiicken. Arch. Schiff. f. Tropenhyg 41: 167-172.

(NS) = not seen