6

Plasmodium cynomolgi Mayer, 1907

IN 1907, Martin Mayer found a malaria in some *Macacus cynomolgus* (= *M. fascicularis)* monkeys imported into Germany from Java and, following a brief description of the parasite, he named it *Plasmodium cynomolgi.* The next year (1908) he described the blood parasites in more detail and was careful to point out the differences between the new parasite and *P. inui* and *P. pitheci,* each described by Halberstaedter and Prowazek in 1907 from the same general area, and between *P. vivax* endemic in the same region. In the ensuing years, up to 1935, when Mulligan again described the parasite and returned it to its original status of Mayer, 1907, the parasite has been reported without a name or under various designations including *P. inui, P. inui* var. *cynomolgi.* However, irrespective of the designation or non-designation, the main characteristics (tertian periodicity, amoeboidity, and Schüffner's stippling, etc.) stand out so clearly as to cause little doubt as to the species involved.

It is indeed fortunate that the strain from Malaya studied by Mulligan has been maintained through the years, and although not the type strain, as pointed out by Eyles (1963), it is the neotype and the one designated as the TC strain (Eyles, 1960a), the M strain (Coatney *et al,* 1961; Schmidt *et al,* 1961), and the Rockefeller strain (Garnham, 1959). In 1959, Garnham gave the subspecific name *bastianellii* to a parasite, also from Malaya, which he thought was enough different to warrant the designation. We have elected to call this the B strain (Coatney *et al,* 1961; Schmidt *et al,* 1961). Each of these strains is discussed more fully later in this chapter.

Inoki *et al* (1942) termed the parasite found in *Macaca cyclopis,* the Taiwan macaque, *P. inui cyclopis.* In 1951, Inoki *et al* added information on its periodicity, 48 hours, which marked it as a tertian parasite rather than a quartan. In line with this information, Garnham (1959) referred to it as *P. cynomolgi cyclopis* and Hsieh (1960) as *P. cynomolgi* var. *cyclopis.* Bray (1963) gave it specific status as *P. cyclopis.* The main point of difference seems to be its virulence which is hardly sufficient reason for specific rank. We propose to wait for more evidence before making a decision regarding the Inoki parasite.

Plasmodium cynomolgi ceylonensis was described by Dissanaike *et al,* (1965) from the Ceylon toque monkey, *Macaca sinica.* The points of difference of this parasite and the neotype are slight. In our studies, we have elected to deal with this parasite as the C strain of *P. cynomolgi.* In the same year, Dissanaike *et al* (1965) mentions a *P. cynomolgi* from the grey *langur (Presbytis entellus thersites).* In our records, we have carried this parasite as the langur strain of *P. cynomolgi.*

In addition, we have studied 6 other isolates of *P. cynomolgi:*

Berok-isolated from a naturally infected *M. nemestrina* monkey trapped in the southern part of the State of Perak, Malaysia. Although the animal was taken in 1961, the isolation was not made until 1964.

PT-isolated from a naturally infected *M. nemestrina* monkey trapped in 1960 in the area of Port Dickson in the State of Negri Sembilan, Malaysia.

RO--isolated from a *M. mulatta* monkey imported into the U.S. from the Assam-Burma area in December, 1960. (Schmidt *et al,* 1961a

and personal communication from Dr. L. H. Schmidt).

Gombak--isolated by Eyles *et al* (1963) from an infected *Anopheles balabacensis introlatus* mosquito taken at Ulu Gombak, near Kuala Lumpur in the State of Selangor, Malaysia.

Cambodian--isolated from a *M. irus* (= *fascicularis)* monkey taken in west-central Cambodia in October, 1962 (Bennett and Warren, 1965).

Smithsonian--isolated by us from a naturally infected stump-tailed macaque, *M. speciosa* (=*arctoides)* monkey housed in the National Zoological Park, Washington, D. C.

Further data on these strains of *P. cynomolgi* are presented later in this chapter.

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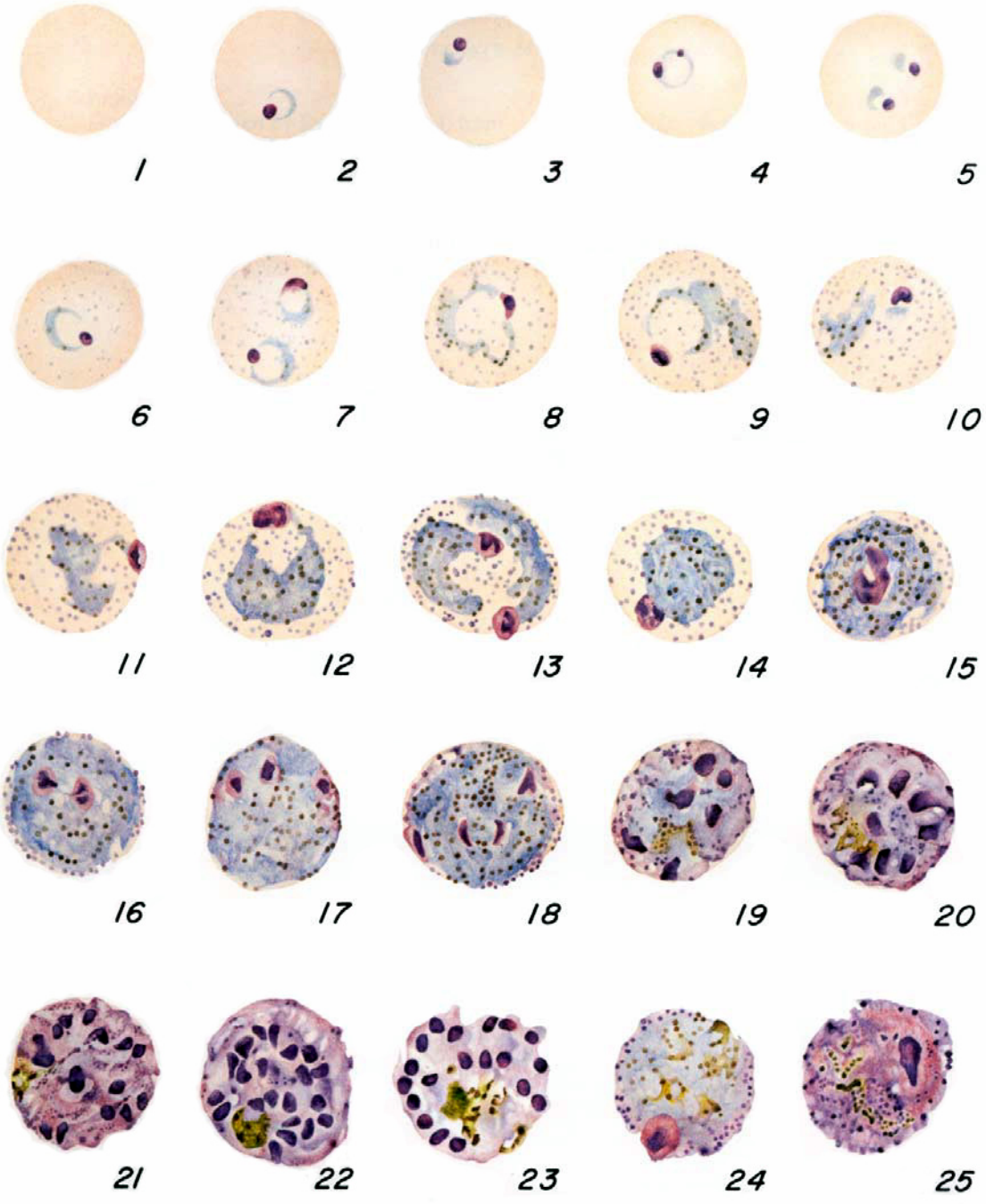


G. H. Nicholson

PLASMODIUM CYNOMOLGI

Cycle in the Blood
PLATE IV

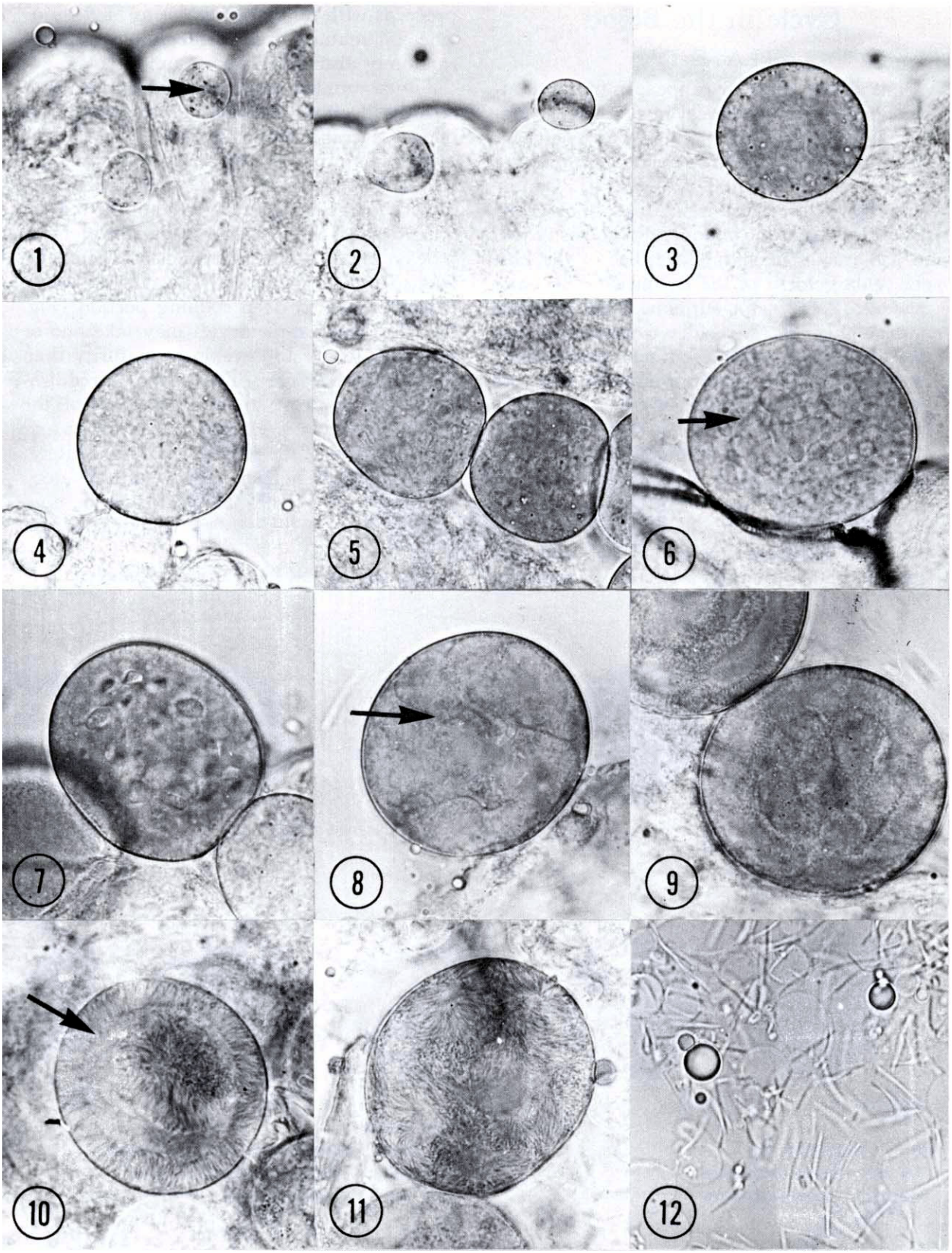
The young ring forms appear in the circulating blood with a spherical nucleus and sometimes with only a whisp of cytoplasm (Fig. 3). The nucleus is generally single but may be double and of unequal size (Fig. 4). Double infection of the red cell is not uncommon (Figs. 5, 7, 13). When the young parasite has grown to a diameter of about half that of the host cell, enlargement of the erythrocyte becomes evident; Schüffner's stippling and pigment appear (Fig. 6). The older trophozoites look like the *P. vivax* parasites of man. The Schüffner's stippling is more prominent and the cytoplasm of the parasite becomes amoeboid (Figs. 8-10); pigment is in small granules, yellowish-brown, and scattered in the cytoplasm. The host cell is now further enlarged. At maturity, the trophozoite almost fills the erythrocyte, its nucleus has increased in size, the parasite appears more compact, and pigment is abundant (Figs. 14, 15). Schizogony proceeds with nuclear divisions as in *P. vivax,* when at maturity the merozoites, which are randomly distributed, may number 14 to 20; the usual number is 16. The pigment becomes condensed either peripherally or centrally in one or several masses (Figs.) 6-23).

The mature gametocytes are round, resemble *P. vivax* forms, and are located in enlarged host cells. The cytoplasm of the macrogametocyte is relatively compact. The pigment is heavy and scattered. The nucleus is compact, located eccentrically, and may have a dense, centrally located deep staining portion (Fig. 24). The microgametocytes may take one or two days longer to develop to maturity than the distaff parasites. They stain a reddish-purple in contrast to the light blue of the macrogametocytes. The nucleus is diffuse and takes up most of the parasite. It may exhibit a more compact portion which stains a deep reddish-black. Pigment is located in the non-nuclear area of the parasite and is scattered (Fig. 25).

The asexual cycle occupies 48 hours.

PLATE IV.—*Plasmodium cynomolgi*

Fig. 1. Normal red cell.
Figs. 2-7. Young trophozoites.
Figs. 8-13. Growing trophozoites, note double infection Fig. 13.
Figs. 14, 15. Nearly mature and mature trophozoites.
Figs. 16-20. Developing schizonts.
Figs. 21-23. Nearly mature and mature schizonts.
Fig. 24. Mature macrogametocyte.
Fig. 25. Mature microgametocyte.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Sporogonic Cycle
PLATE V

Garnham (1966) stated that the microgametocyte of *P. cynomolgi* exflagellates very readily, producing about 8 microgametes. The nuclei of the male and female elements fuse to form the zygote within 8 hours of fertilization producing a very condensed body measuring only 4 μ in diameter. The zygote assumes a vermiculoid shape and thus becomes an ookinete at about 16 hours. The hind portion is swollen and the front portion is narrowed almost to a point. At 24 hours, it is 16 μ long. The dark golden brown pigment grains are collected in strands at the broad end, while a small vacuole is present near the tip. Penetration of the gut epithelium to become the oocyst is completed in 40 to 42 hours after the infective feeding.

Using phase-contrast microscopy and time lapse photography, Freyvogel (1966) observed that the ookinete of *P. cynomolgi* travels with a spiral-type motion. The ookinete is able to anchor itself to a surface with its posterior (blunt) end.

In describing the early changes in the nucleus, Bano (1959) noted that before 48 hours, the nucleus is in a resting stage. The spireme then gives rise to eight diploid chromosomes, two pairs of which are large and filamentous and two pairs are small and bead-like. The binuclear oocyst is formed by the 50th hour when the oocyst is about 14 μ in diameter. The pigment granules are scattered in a linear pattern throughout the oocyst. After 72 hours, the nuclei, the majority of which are at the resting phase, number 10 to 12 in each oocyst. Green (1932) was apparently the first to describe the development of *P. cynomolgi* in the mosquito. In *Anopheles kochi,* 8 days after feeding, the oocysts had an average diameter of 47 μ; on the 9th day, the average diameter was 77 μ and oocyst differentiation was apparent. Sporozoites were present in the salivary glands by day 14.

Extensive studies on the growth of *P. cynomolgi* in different mosquitoes were made by Bennett *et al* (1966a, 1966b). Employing 5 different strains of the parasite, they demonstrated that there were strain differences in the average size of the mature oocysts as well as in the time required for the sporozoites to reach the salivary glands. Such differences occurred with the same strain of the parasite in different species of mosquitoes and were, therefore, considered characteristic of the plasmodium and not dependent on the host mosquito. Only the success of the oocysts maturing and of the sporozoites reaching the salivary glands were controlled by the species of mosquito. Using the median oocyst size, on the day of maturation, as a basis for comparison of the strains, the M strain was the largest (60 to 70 μ), followed by B (50 to 60 μ), Gombak (45 to 50 μ), Berok (40 to 45 μ), and, finally, Cambodian (35 to 50 μ). When a comparison of the strains was made with regard to the day sporozoites were first present in the salivary glands, they found that the M strain took the longest (9.5 to 10.5 days) followed by B (9.5 days), Gombak (9.5 days), Berok (7.5 to 8.5 days), and the Cambodian (7.5 days). Sporozoites were found in the salivary glands in the shortest time in the parasite producing the

PLATE V

FIGURES 1-12.—Developing oocysts and sporozoites of *Plasmodium cynomolgi* (B strain) in *Anopheles b. balabacensis* mosquitoes X 580 (Except Figures 1, 3, and 12).

Fig. 1. 4-day oocysts showing scattered pigment. X 740.
Fig. 2. 5-day oocysts.
Fig. 3. 6-day oocyst. X 740.
Fig. 4. 7-day oocyst.
Fig. 5. 7-day oocysts.
Fig. 6. 8-day oocyst showing first signs of differentiation.
Fig. 7. 8-day oocyst showing early differentiation.
Fig. 8. 9-day differentiating oocyst.
Fig. 9. 9-day oocyst showing more advanced stage of differentiation.
Fig. 10. 10-day differentiated oocyst.
Fig. 11. 10-day fully differentiated oocyst.
Fig. 12. Sporozoites near salivary gland tissue. X 930.

smallest oocysts. These workers concluded that studies on the sporogony of the different species of malaria and isolates might contribute to a better understanding of the relationships between species and between different isolates, too.

Differences in oocyst diameters between different strains of *P. cynomolgi* were also emphasized by Dissanaike *et al* (1965) when they indicated that by day 7, oocysts of the B and C strains had an average size of 50 µ whereas the M strain had a mean size of only 27 µ.

In our studies, only the B strain was studied in any detail (Table 3). This parasite was readily infectious to mosquitoes and sporogony was normal in each of the 5 species of *Anopheles* tested. In *A. freeborni,* the oocysts 4 days after feeding had a mean diameter of 14 µ with a range of 9 to 18 µ. The oocysts continued to grow so that by day 10, the mean oocyst diameter was 61 µ with a range of 41 to 89 µ. Sporozoites were present in the salivary glands by day 11.

The oocyst diameters in the other 4 species were within the range found in *A. freeborni*. Sporozoites were present in the salivary glands of the *A. b. balabacensis* and the *A. stephensi* on day 10, and in the *A. maculatus* and the *A. quadrimaculatus* on day 11. A comparison of the oocyst growth curves (Fig. 13) in *A. freeborni* and *A. b. balabacensis* shows that in the *A. b. balabacensis* the oocysts were slightly larger and that the sporozoites appeared in the salivary glands one day sooner than in *A. freeborni.* This conflicts slightly with the findings of Bennett *et al* (1966a) who found that the sporozoites were in the salivary glands sooner with the parasite producing the smaller oocysts.

According to Bennett *et al* (1966a), the sporozoites of the Cambodian strain of *P. cynomolgi* averaged 9 to 10 µ in length and 1 to 2 µ in width. The nuclei showed a variety of forms which they divided into three categories: 1) the nucleus diffuse, occupying one-third to one-half of the sporozoite, 2) the nucleus in two or three compact, dense, adjoining fragments, and 3) the nucleus in a single, compact, dense mass occupying one-fifth to one-fourth of the body and situated about midway in the sporozoite. The average length of the sporozoites gradually increased between the first and seventh day of presence in the salivary glands. Category 3 forms, were predominant throughout the period of observation although category 1 forms were well represented on the first 2 days and category 2 forms on the first 3 days. By day 7 to 9, 98 to 100 percent of the sporozoites belonged to the third category.

Employing electron microscopy, Garnham *et al* (1963) showed that the pellicle of the sporozoite is about 25 mµ thick and appears as two electron-dense layers separated by a less dense zone.

TABLE 3.–Oocyst diameters of *Plasmodium cynomolgi* (B strain) in *Anopheles freeborni, A. b. balabacensis, A. maculatus, A. stephensi and A. quadrimaculatus.*

Days after Infection	*A. freeborni*			*A. b. balabacensis*			*A. maculatus*			*A. stephensi*			*A. quadrimaculatus*		
	No.	Range	Mean*	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
4	100	9-18	14	100	8-18	14	107	8-17	13	100	11-18	14	105	12-17	14
5	118	9-19	14	116	12-19	15	127	11-19	14	133	11-21	15	131	12-25	19
6	115	12-35	23	113	14-31	22	138	12-27	21	114	12-30	22	120	12-30	22
7	105	18-53	35	147	22-55	38	130	12-38	25	111	12-37	25	107	14-47	30
8	127	17-59	42	125	27-61	47	103	18-53	38	124	24-66	45	135	18-65	43
9	149	24-83	53†	100	30-84	57†	125	21-84	55†	125	21-74	52†	120	35-83	62†
10	103	41-89	61†	123	30-94	68†‡	118	24-67	50†	100	30-74	57†‡	125	29-92	64†
11	95	28-89	56†‡	134	20-92	60†‡	122	24-77	48†‡	105	30-77	56†‡	126	24-94	52†‡

* Measurements expressed in microns; incubation temperature 25° C.
† Oocyst differentiation.
‡ Ranges in oocyst diameters not available.

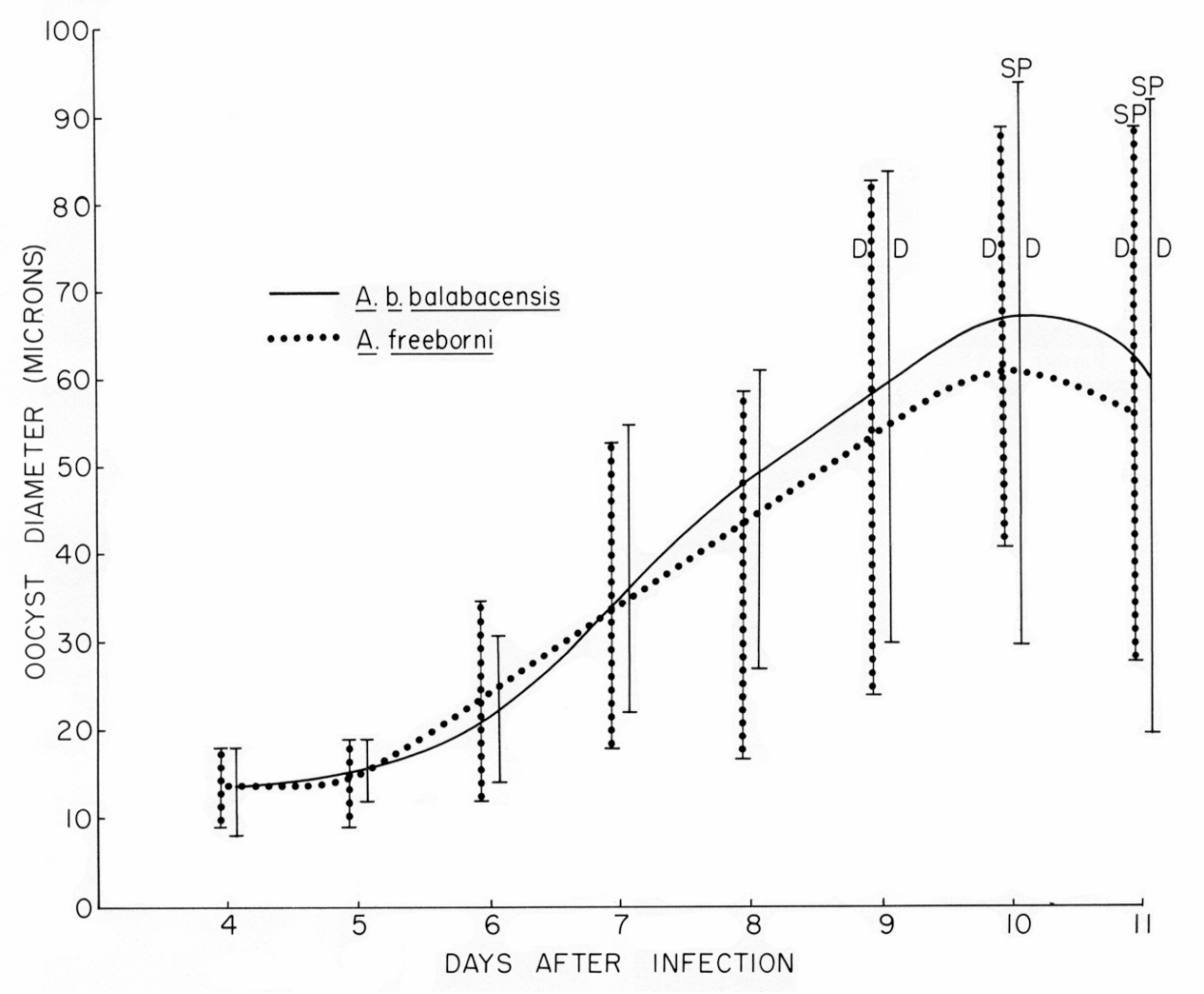
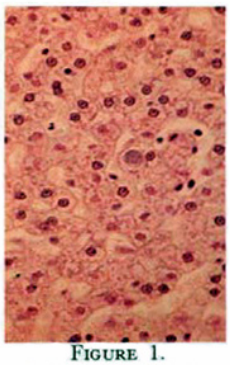


FIGURE 13.—A comparison of the mean oocyst diameter curve and ranges in oocyst diameters of *Plasmodium cynomolgi* in *Anopheles b. balabacensis* and *A. freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).



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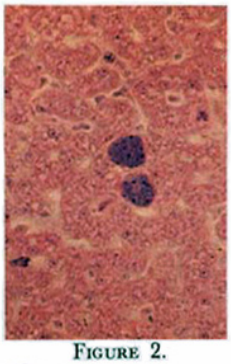

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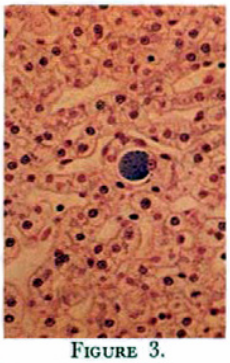

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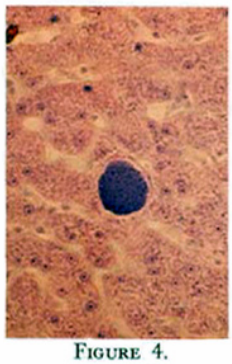

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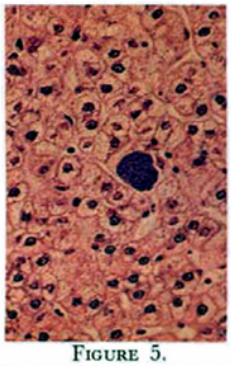

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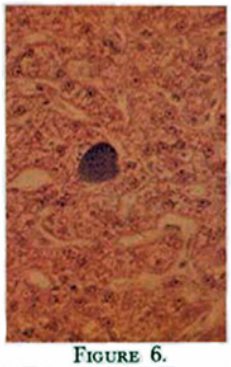
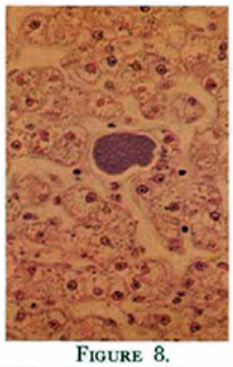


FIGURE 6.

FIGURE 7.



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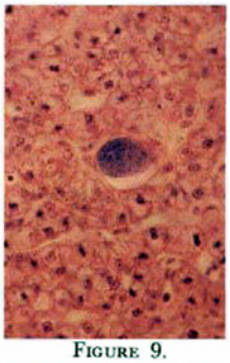

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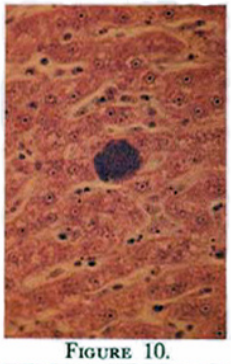

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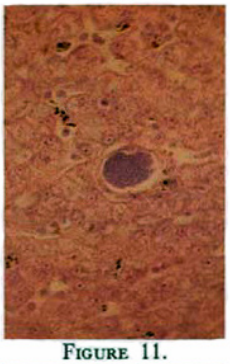

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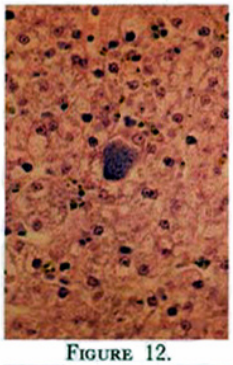

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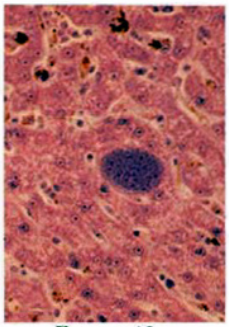

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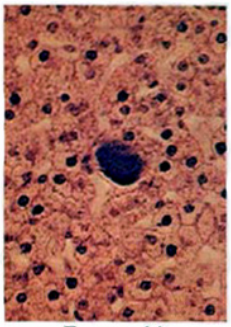

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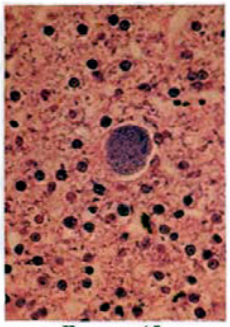

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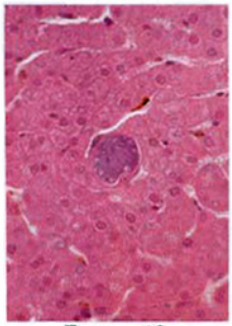

FIGURE 16.

Cycle in the Tissue
PLATE VI

The discovery of an exoerythrocytic stage in primate malaria was announced by Shortt and Garnham on 24 January, 1948. Prior to the actual discovery it was agreed generally that contrary to Schaudinn's convincing "dream", of the sporozoite going directly into the host red cell, there had to be a fixed tissue stage to account for prepatent periods and for relapses. In fact, in speaking of the latter, Thayer (1897) in a series of lectures on human malaria, at the Johns Hopkins University, agreed with earlier investigators that there must be some undiscovered form of the parasite; he wrote "the organism may remain perhaps within the cell body of certain phagocytes for long periods of time, only to be set free again as a result of some insult, the nature of which is not yet appreciable to us." Fifty-one years later, it did become "appreciable to us." In the interim, pre-erythrocytic stages of many of the bird malarias were described, mainly by the Huff school of investigators (see Huff and Coulston, 1944 and Huff, 1947). Also, during this period, there were various doubtful records of a corresponding stage in human malaria (see Angelini, 1947 and Huff, 1947).

Fairley *et al* (1945, 1947) transferred from 200 to 500 ml of blood from patients bitten by infected mosquitoes to clean test subjects. Recipients became infected when blood was taken within 30 minutes of mosquito biting but after that, all results were negative until day 7 in falciparum infections, and day 8 in vivax infections. The evidence was clear, that wherever the sporozoites and subsequent schizogonies occurred, it was not in the peripheral blood. Similarly, our own studies carried out in March of 1945 (Cooper, Ruhe, and Coatney, 1949) showed that with the St. Elizabeth strain of *P. vivax* the blood is not infectious during the latent period in patients whose infections relapsed subsequently. These authors transferred 250 ml. of blood from patients 181 days after the primary infection to other volunteers. Only one of 6 recipients developed malaria and his donor experienced a relapse 7 days after blood was drawn for transfer. The other 5 volunteers were shown to be susceptible to infection when homologous strain parasitized blood was inoculated into them. These and similar experiments did not prove the presence of EE stages in primate malaria but as indirect evidence the stage was set for the Shortt and Garnham discovery.

The initial announcement told of finding 6- and 7-day EE bodies and subsequent papers through 1954 (Shortt and Garnham, 1948, 1948a; Shortt, Garnham, and Malamos, 1948; Hawking, Perry, and Thurston, 1948; Shortt, 1948; Shortt and Garnham, 1948b; and Shortt, Bray, and Cooper, 1954) filled in the primary exoerythrocytic schizogony from day 2 through day 12 and certain older forms including one at day 104.

As shown by Fairley *et al* (loc. cit.) the sporozoite leaves the peripheral blood shortly after it is introduced into the host and probably enters a parenchyma cell of the liver. The

PLATE VI.—Exoerythrocytic bodies of *Plasmodium cynomolgi* in liver of *Macca mulatta* monkeys. X 240. Figs. 1-5, 11, 12, 14-16 are B strain; Figs. 6, 10, and 13 are M strain; Figs. 7 and 9 are RO strain; Fig. 8 is PT strain. Figs. 1-15 EE bodies stained by Giemsa-Colophonium technique; Fig. 16 EE body stained with Hematoxylin-Eosin.

Fig. 1. 5 day body.
Fig. 2. 6 day bodies showing prominent flocculi.
Fig. 3. 6 day body showing host cell nucleus.
Fig. 4. 7 day body.
Fig. 5. 7 day body.
Fig. 6. 8 day body.
Fig. 7. 9 day body.
Fig. 8. 10 day body showing numerous nuclei.
Fig. 9. 11 day body.
Fig. 10. 12 day body.
Fig. 11. 14 day body.
Fig. 12. 17 day body.
Fig. 13. 18 day body.
Fig. 14. 60 day body.
Fig. 15. 105 day body.
Fig. 16. 143 day body; this figure courtesy of Dr. Leon Schmidt.

earliest stage seen, a 2-day form, is a spherical to ovoid body about 2.3 to 2.45 µ in diameter. The cytoplasm stains blue; the nucleus is single and stains red. (The authors were not unmindful of the difficulties connected with recognizing this minute stage and that of day 3, also; however, their illustrations appear convincing.) The 3-day form is spherical or ovoid and measures 4.5 to 5.9 µ in diameter. It occupies a more or less peripheral position in the cell. The cytoplasm stains blue and there are 8 or more nuclei which stain reddish-violet. The 4-day forms are about twice the size of those of the previous day, about 10 µ in diameter. The cytoplasm stains a pastel blue. The nuclei have increased to about 20 and stain a reddish-mauve. The periphery of the schizont is marked by a fine membrane. By the next day, the 5th, the schizont is about 14 µ in diameter and houses up to 70 nuclei. The host cell nucleus is pushed to one side but otherwise the host cell shows no sign of being invaded; staining is typical.

Up to this point, the development of the tissue parasite is more or less routine but by the 6th day there is a decided change. The size is now up to 29 µ in diameter, the host cell is much enlarged, and its nucleus is pushed toward the periphery. The cytoplasm of the parasite stains a pastel blue. The nuclei are difficult to count but the number is over 100. Vacuoles are a new development and become more prominent as growth proceeds. Seven day forms may assume various shapes, other than the characteristic oval, due to tissue resistance, but irrespective of the shape the internal structure is constant. The cytoplasm stains light-blue or mauve and is somewhat granular. The chromatin particles stain magenta, tend to be spherical, rod- or boat-shaped and measure about 0.75 µ in diameter. The 8-day EE bodies average about 38 µ in diameter. They are spherical to oval. The tendency for vacuolization seems to be a feature connected with parasites from some monkeys and absent from others. The cytoplasm stains blue as in the younger forms, the nuclei red. Some of the schizonts are mature by this time because parasites may appear in the peripheral blood. By the 9th day, the maturation of the EE bodies is definitely in force but since there is a lack of complete synchronicity, remaining first generation schizonts continue their development. These slower-growing forms are compact and entire. The merozoites appear as if composed entirely of chromatin but there is surely a cytoplasmic envelope, too. Bray (1957) found these forms measured 46 µ in diameter and Eyles (1960a) found them to measure about 49.8 µ with extremes of 35 to 62.5 µ. Ten day forms generally contain differentiated merozoites. Ruptured forms, when seen, appear to have lost their compact regular outline. Older first generation schizonts have been seen but except for their large size, up to 108 µ, they appear about like the 8 to 10 day forms.

Secondary schizonts have been seen at 60 and 105 days (see Plate VI, Figs. 15 and 16) 143 days by Schmidt and at 378 days by Warren. Secondary schizonts is an arbitrary designation for there is no proof that these older forms, probably responsible for relapse, arise from the rare parasite of the first or succeeding generations and, for reasons of their own, re-enter a liver cell. Certainly these liver schizonts do not originate from circulating blood forms--for if they did, blood-induced infections would relapse, also. They do not. Relapse, in the true sense, is a part of the life-pattern of *P. cynomolgi* as it is of *P. vivax, P. fieldi,* and certain others, but the process responsible for producing the tissue schizonts which initiate it is still a matter for speculation. It is not impossible that these continuing EE bodies are derived from the original sporozoites which lie dormant for a time, and then, through some urge, not appreciable to us, are moved to complete their destiny. A fuller discussion is found in an earlier section (Chapter 4).

The secondary schizonts, found at 60 days and/or later, resemble the initial forms in all respects including active division. This latter characteristic shows that they are not latent or retarded forms. Garnham (1966) believes that these forms can be separated from earlier forms because their contour is generally "markedly convoluted." This has not been our experience as may be seen by examining Figs. 15 and 16 (60- and 105-day parasites) and the same is true for the 143- and the 378- day forms. As the

evidence stands now we doubt that the contour of the parasite can be relied upon as an indication of the age of the EE body.

There were no reports on the ultrastructure of the EE bodies of any of the primate malarias until the paper by Sodeman *et al* (1970) on 7-day forms of the B strain of *Plasmodium cynomolgi* (Plate VII). They studied 4 of these bodies which had an average size of 28 x 17μ. The liver cell border, with its organelles, surrounds the EE body completely. The host cell is enlarged and its nucleus is pushed to one side. There appear to be no degenerative changes in the cell as a result of the parasitism.

The lobulated EE body has a two-layered membrane which lies close to the cytoplasm of the parasite. Mutliple irregularly shaped nuclei, 1.1 x 1.6 μ, are scattered throughout the body. The nucleus is homogenous and granular with a clear space surrounding it. The cytoplasm of the EE body contains densely packed free ribosomes. Some of these appear in a linear fashion. Mitochondria are present.

There are 2 types of vacuoles: Type 1 is 0.3 to 3.5 μ, round, and with a distinct membrane. They appear empty but some contain membrane-like structures; Type 2 vacuoles are smaller, homogeneous, and more electron dense. They are generally round, but may be crescent-shaped.

It is hoped that these authors will continue

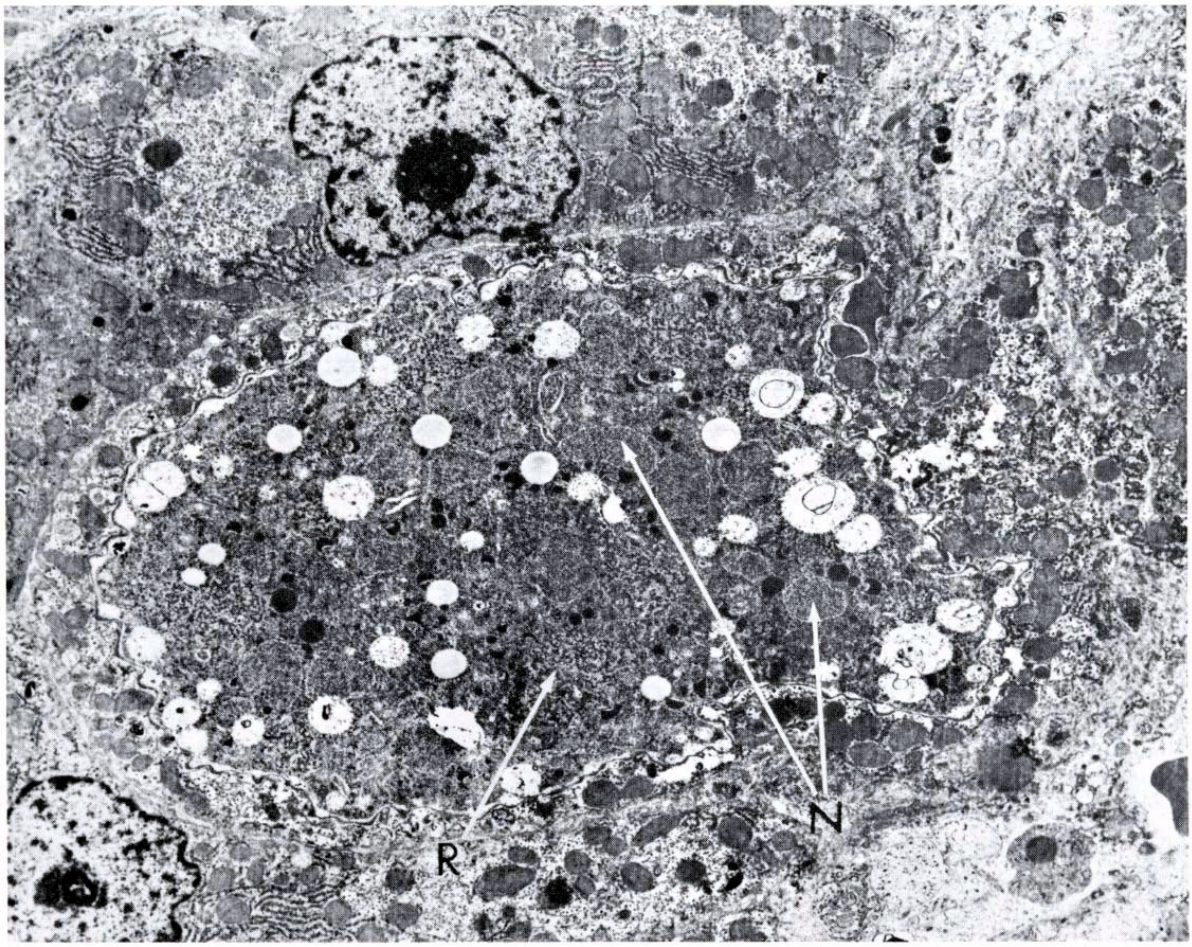

PLATE VII.—Midsection of a 7-day-old exoerythrocytic body of *Plasmodium cynomolgi* in rhesus monkey liver. The parasitized hepatic cell surrounds the EE body. A wavy outer membrane and a thin inner membrane enclose the EE body. Several areas of linear arranged ribosomes are seen (R). Nuclei (N) have a clear space surrounding them. (X 4600). Electron-photomicrograph courtesy of Dr. Thomas Sodeman.

their investigations so that we may learn more about the ultrastructure of this and other primate malaria EE bodies.

Course of Infection

Plasmodium cynomolgi is readily transmitted by a number of species of mosquitoes. In our studies, there was a total of 132 successful transmissions out of 136 attempts (Table 4). These transmissions were initiated by the inoculation of sporozoites, by mosquito bite, or by intravenous and/or intrahepatic injection. The prepatent periods ranged from 7 to 16 days with a mean of 9.8 days. In general, slightly shorter prepatent periods were obtained when large numbers of sporozoites were inoculated intravenously and/or intrahepatically than were obtained by mosquito bite.

The two strains of *P. cynomolgi* which have received the greatest attention are the Mulligan or M strain and the subspecies *bastianellii* or the

TABLE 4.—Summary of transmissions for seven strains of *Plasmodium cynomolgi* using six species of *Anopheles.*

Strain	Transmissions/Attempts						Total trans.	Prepatent Period (days)	
	Fre*	Qua	Ste	Mac	Atr	Bal		Range	Mean
B†	10/10	23/23	1/1	44/47		8/8	86/89	7-16	9.8
M		2/2		4/4			6/6	8-11	10.0
Camb	2/2	1/1					3/3	9-13	11.0
Berok	1/1	6/6		2/2			9/9	8-12	9.7
Gombak	1/2	1/1	1/1	2/2		1/1	6/7	8-14	10.0
RO	2/2	11/11	1/1		1/1		15/15	8-12	9.4
C	3/3	1/1	1/1	2/2			7/7	8-16	10.7
Totals	19/20	45/45	4/4	54/57	1/1	9/9	132/136	7-16	9.8

* Fre = *Anopheles freeborni*, Qua = *A. quadrimaculatus*, Ste = *A. stephensi*, Mac = *A. maculatus*, Atr = *A. atroparvus*, Bal = *A. b. balabacensis*.
† B = Bastianellii strain, M = Mulligan strain, Camb = Cambodian strain, Berok = Berok strain, Gambok = Gambok strain, RO = Rossan strain, C = Ceylonensis strain.

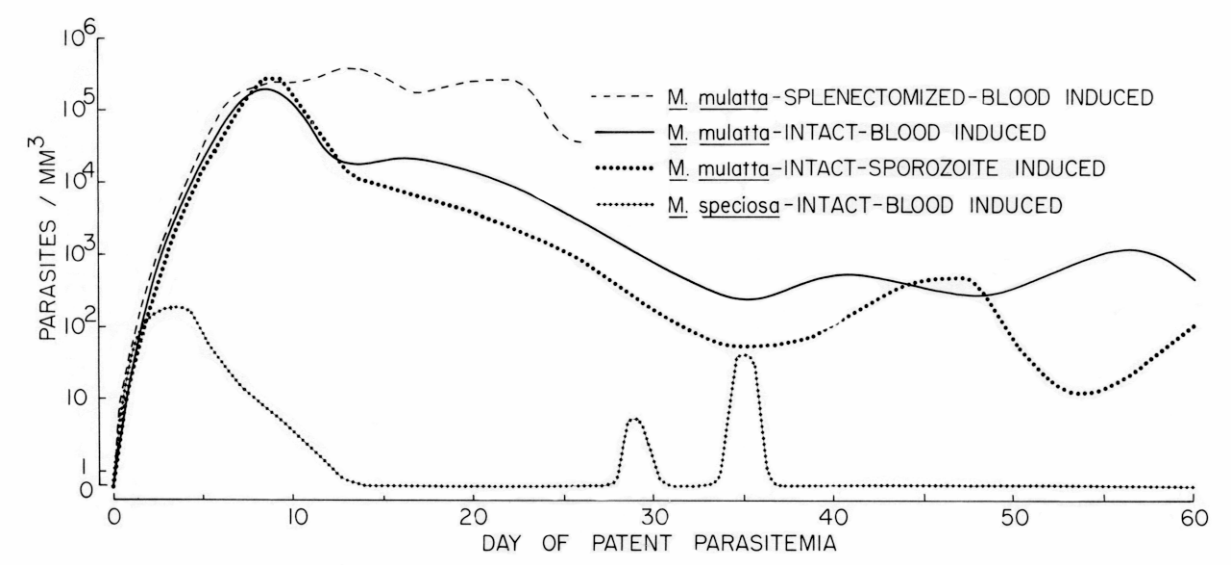

FIGURE 14.—Parasitemia curve for 1 *Macaca speciosa* and median parasitemia curves for 102 *M. mulatto* monkeys infected with the B strain of *Plasmodium cynomolgi*.

B strain. The histories of these two isolates were ably discussed by Eyles (1963).

Our efforts have been primarily directed towards study of the B strain, the results of which are shown in Figure 14. In 8 splenectomized *M. mulatta* monkeys infected by inoculation of parasitized blood, the median parasitemia reached a level of approximately 200,000 per mm^3 by day 8, followed by rises to approximately 400,000 and 300,000 per mm^3 on days 13 and 22, followed by a sudden drop in parasite levels. Maximum parasitemias as high as 1,200,000 were obtained in some animals during the 26 day observation period. In 44 intact *M. mulatta* monkeys similarly inoculated, the peak median parasitemia of approximately 200,000 per mm^3 was obtained on day 8, also. However, in these animals, the parasitemia fell rapidly to a level of 18,000 per mm^3 by day 13 and after several succeeding fluctuations, reached a level of approximately 500 per mm^3 by day 60. In 50 intact *M. mulatta* monkeys, infected by inoculation of sporozoites, the peak median parasitemia was about the same as that encountered in the blood-induced infections. However, the parasitemia dropped to a lower level by day 35 (50 per mm^3) followed by a rise in parasite level by day 47 and, then, by another drop in the parasitemia. The median parasite count after 60 days was only 100 per mm^3. The inoculation of one *M. speciosa* monkey resulted in a peak parasitemia of 200 per mm^3, 4 days after inoculation. This was followed by a drop to an undetectable level. Two recrudescenses were observed 28 and 35 days after inoculation.

In order to examine the normal parasitemias of infections with both the M and the B strains, Dr. Leon Schmidt of the Southern Research Institute, Birmingham, Ala., supplied the data, from 174 *M. mulatta* monkeys, used in preparing Figures 15 through 19.

The B strain infections, induced by the inoculation of parasitized blood (Fig. 15), gave a mean peak parasitemia, by the 8th day of patency, of approximately 1,000 per 10,000 RBC. After the peak, the parasite level rapidly declined to approximately 80 per 10,000 RBC by day 12. This was followed by successive decreasing waves in the parasite count until day 45 when the parasitemia had fallen to 2.6 per 10,000 RBC. Thereafter, the parasitemia rose and was 10 per 10,000 RBC at the end of the 60 day observation period. In the monkeys infected by inoculation of sporozoites (Fig. 16), the peak mean parasitemia was again, on the 8th day of patency, at a level of approximately 700 per 10,000 RBC. The parasite level then declined

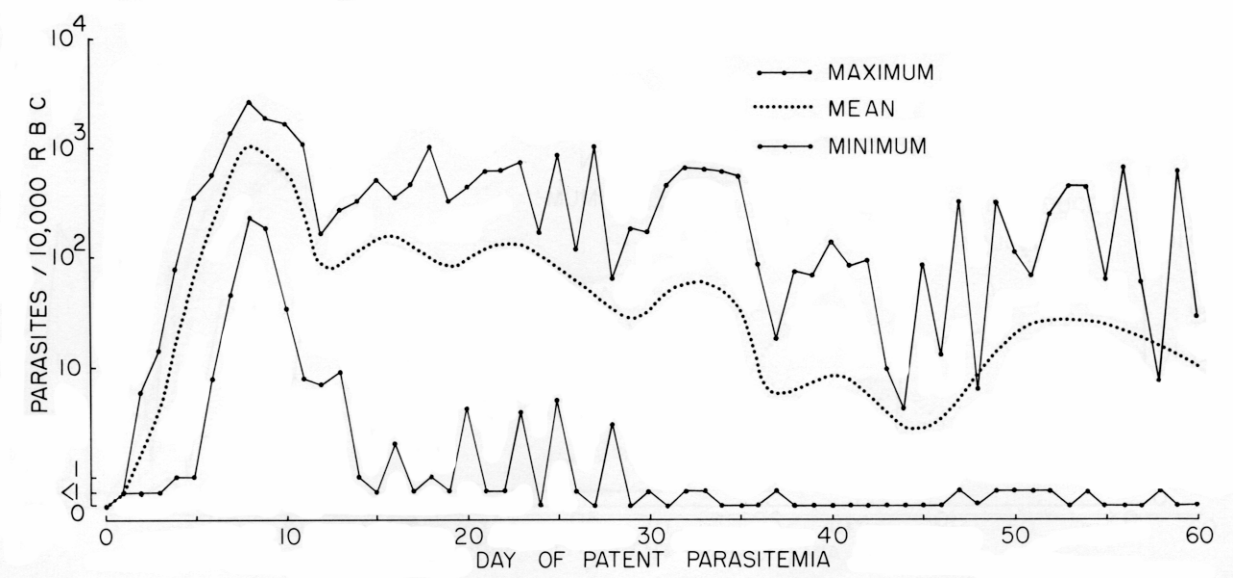

FIGURE 15.—Mean parasitemia curve, with minimum and maximum parasite counts, for 29 *Macaca mulatta* monkeys infected with B strain *Plasmodium cynomolgi* by the inoculation of parasitized blood. (Data courtesy Dr. Leon Schmidt).

rapidly to approximately 90 per 10,000 RBC on day 14. This was followed by a declining parasite count to a level of approximately 5 per 10,000 RBC by day 60.

The M strain infections, initiated by the inoculation of parasitized blood (Fig. 17) resulted in a peak mean parasitemia on day 9 with a count of approximately 600 per 10,000 RBC. The parasite level declined rapidly to a much lower level than encountered with the B strain, approximately 4 per 10,000 RBC by day 23. The mean parasitemia remained at this low level for the remainder of the 60-day observation period. In monkeys infected with the M strain, by sporozoite inoculation (Fig. 18), the peak mean parasitemia was on day 10 at a level of

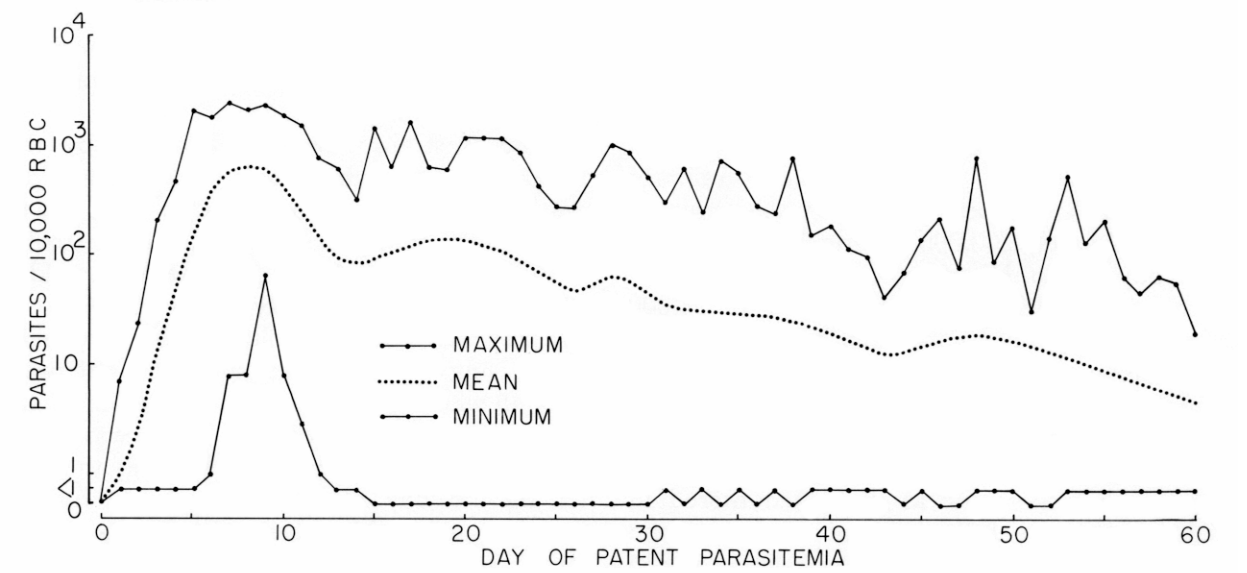

FIGURE 16.—Mean parasitemia curve, with minimum and maximum parasite counts, for 60 *Macaca mulatta* monkeys infected with B strain *Plasmodium cynomolgi* by inoculation of sporozoites. (Data courtesy Dr. Leon Schmidt).

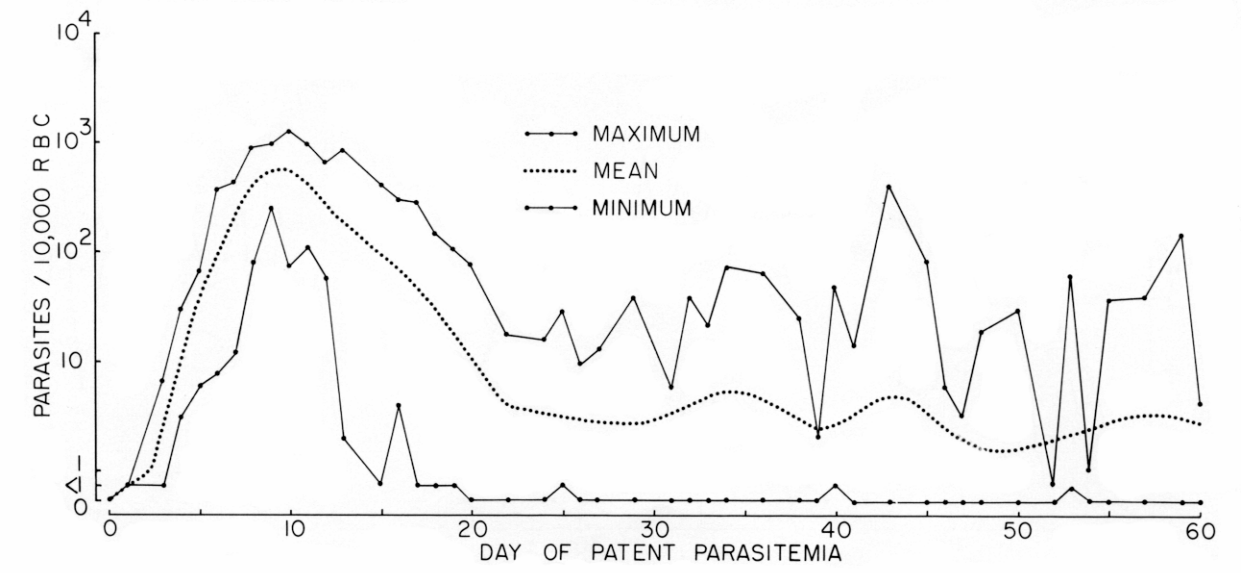

FIGURE 17.—Mean parasitemia curve and minimum and maximum parasite counts, for 25 *Macaca mulatta* monkeys infected with M strain *Plasmodium cynomolgi* by the inoculation of parasitized blood. (Data courtesy Dr. Leon Schmidt).

approximately 500 per 10,000 RBC. The parasite level then declined to approximately 1.4 per 10,000 RBC by day 30. After a subsequent rise, the parasitemia eventually fell to approximately 1 per 10,000 RBC by day 60.

As shown in Figure 19, sporozoite-induced infections with the B strain peak slightly sooner than do those with the M strain parasite. The main difference between the 2 isolates lies in the subsequent parasite levels. After day 15, the B strain maintains a consistently higher level of parasitemia throughout the 60-day observation period. That this characteristic is attributable to this particular strain is evidenced by the fact that the same difference in the parasitemia occurred with animals infected by blood inoculation. Only in the period between day 35 and day 50, when the curve of the blood induced infections with the B strain dropped, was there a joining of the curves for the 2 strains. It appears clear that the

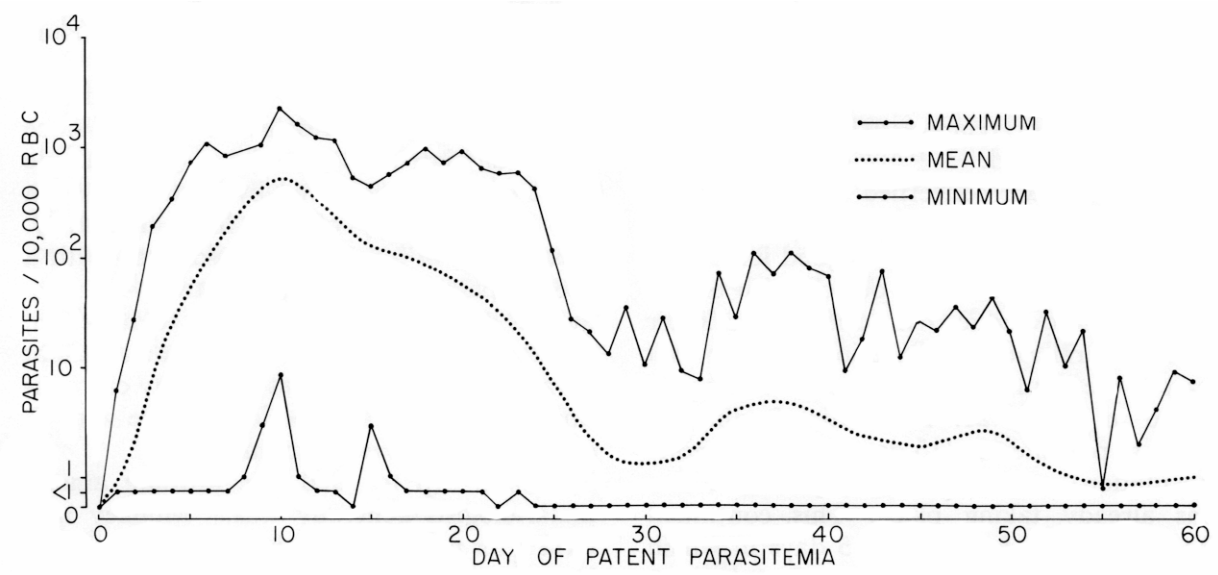

FIGURE 18.—Mean parasitemia curve and minimum and maximum parasite counts for 60 *Macaca mulatta* monkeys infected with M strain *Plasmodium cynomolgi* by inoculation of sporozoites. (Data courtesy of Dr. Leon Schmidt).

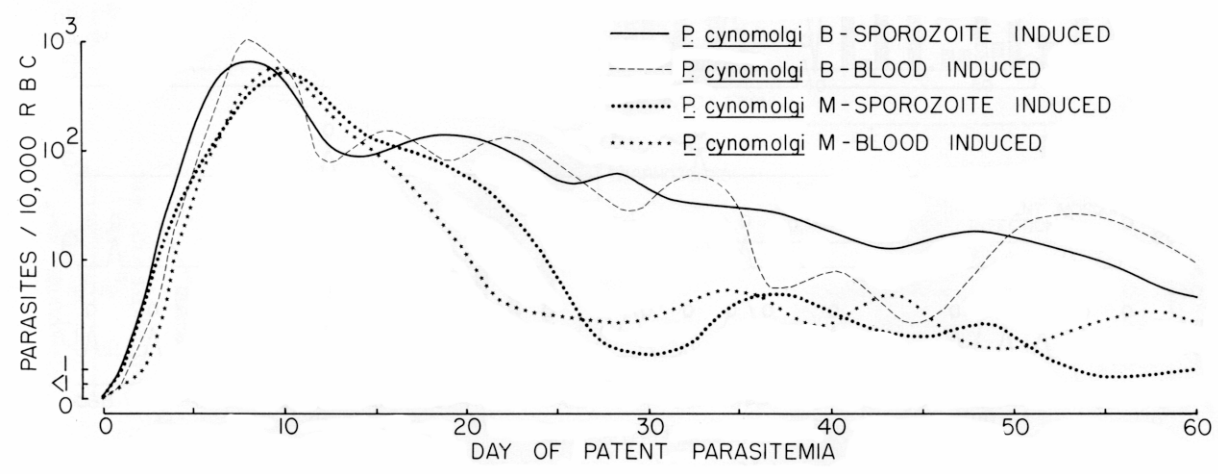

FIGURE 19.—Mean parasitemia curves for infections of B strain *Plasmodium cynomolgi* and M strain *P. cynomolgi* in *Macaca mulatta* monkeys.

B strain parasitemias were consistently maintained at a higher level than were those of the M strain.

It has long been established that *P. cynomolgi* is a relapsing malaria in the true sense. In monkeys infected with the M strain, via mosquito bite, during a one-year period of observation, there were from 2 to 17 relapses (Fig. 20). In one monkey (T-445), the appearance of relapses was frequent and, in some ways, predictable; in another (T-495), relapses occurred only twice, 40 and 120 days after exposure to infection. The total number of relapses which an animal, such as T-445, is capable of having would appear to be large since there appeared to be little diminution in frequency of relapses at the end of the observation period.

We have been concerned for a long time about the most propitious time for feeding mosquitoes in order to obtain good infections. Studies have been carried out on both blood- and sporozoite-induced infections. The latter is the natural mode of infection and, for that reason, the results of one of the studies is presented in Figure 21. The initial feeding on day 5 of parasitemia (15 days after sporozoite inoculation) revealed that mosquito infection was already taking place. This continued through day 36 followed by 5 days in which mosquitoes failed to become infected. Subsequent mosquito infections appeared to be correlated with rises in the parasitemia (relapses or recrudescenses of the infection). Of considerable interest was the presence of a pattern of every-other-day infectivity which persisted often for many days; for example, between days 26 and 37, days 42 and 63, and days 78 and 89. This pattern of infectivity to mosquitoes was also seen in similar studies with other *P. cynomolgi* infections. Since the highest level of infection was correlated with the predominance of very young trophozoite forms in the blood, it is postulated that the gametocytes mature at approximately the same time as the schizonts; and, that they are more infectious in this early period than 24 hours later. It appears that gametocytes lose their infectivity fairly rapidly. Whether this infectivity is subsequently restored is doubtful since a day of high level infectivity (for example day 34) may be followed by several days (in this case 7 days) of low level infectivity. Restoration of infectivity is associated with a rise in parasitemia. The infectivity to mosquitoes 113 days after exposure to infection, or, after 103 days of almost continuous patent parasitemia, indicates that infectivity can continue for an extended period of time with *P. cynomolgi* in *M. mulatta* monkeys.

In man. Up to 1960, the attitude among malariologists generally was: "Monkey malaria is for monkeys, and human malaria is for humans." That attitude took a shattering blow, on 5 May 1960 when the senior author (GRC), on answering a telephone call from Dr. Don E.

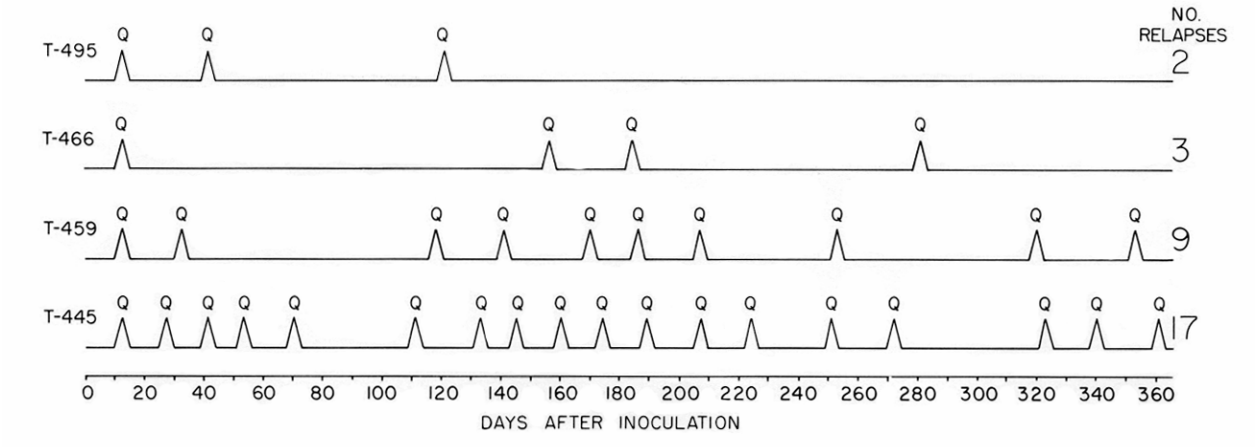

FIGURE 20.—Frequency of relapse activity in 4 *Macaca mulatta* monkeys infected with M strain of *Plasmodium cynomolgi* by the inoculation of sporozoites via the bites of *Anopheles maculatus* mosquitoes. (Drug regimen: 300 mgm. quinine x 5 days with each appearance of parasites).

Eyles, head of the Section on Cytology, Laboratory of Parasite Chemotherapy, NIB, in Memphis, Tennessee, heard him say, "Bob, I have monkey malaria." I was incredulous--had it really happened? The remainder of the conversation went about as follows:

GRC: If you have monkey malaria, don't take any drugs.

DEE: I thought you would say that so I took chloroquine before I placed this call to you.

GRC: I hope you drew blood for inoculation into a clean rhesus.

DEE: I did, and the blood has been given to the monkey. Now all we have to do is wait for the monkey to come down.

(It did-8 days later: Eyles *et al,* 1960).

This was the first recognized instance of a simian malaria infection transmitted to man by natural means and, therefore, what happened during the next two months was a prelude to the developments of the next 10 years.

Dr. Eyles' illness was due to an accidental infection in connection with the study of the effect of drugs on the exoerythrocytic stages of the B strain of *P. cynomolgi* (Eyles and Coatney, 1962). Because it was thought, "man could not be infected with monkey malaria" and because the object was to get sporozoites from as many mosquitoes as possible during a single day, Dr. Eyles and his technician (Mrs. N.C.O.) paid scant attention to the occasional mosquito that escaped into the room. Two days after Dr. Eyles recognized the cause of his illness, 7 May, the technician (N.C.O.) became ill with fever. Five ml. of her blood was given to a clean rhesus monkey; parasites were present 10 days later, and a normal infection ensued. On the same day, another 20 ml. of blood was drawn from N.C.O. and divided between two inmate volunteers. Each of the volunteers developed clinical malaria, but neither one had anything but minimal parasite counts.

At the time of the subinoculations, there was no proof that the parasite involved was *P. cynomolgi* and, if it were *P. cynomolgi,* that it was transferred by mosquito bite. To answer

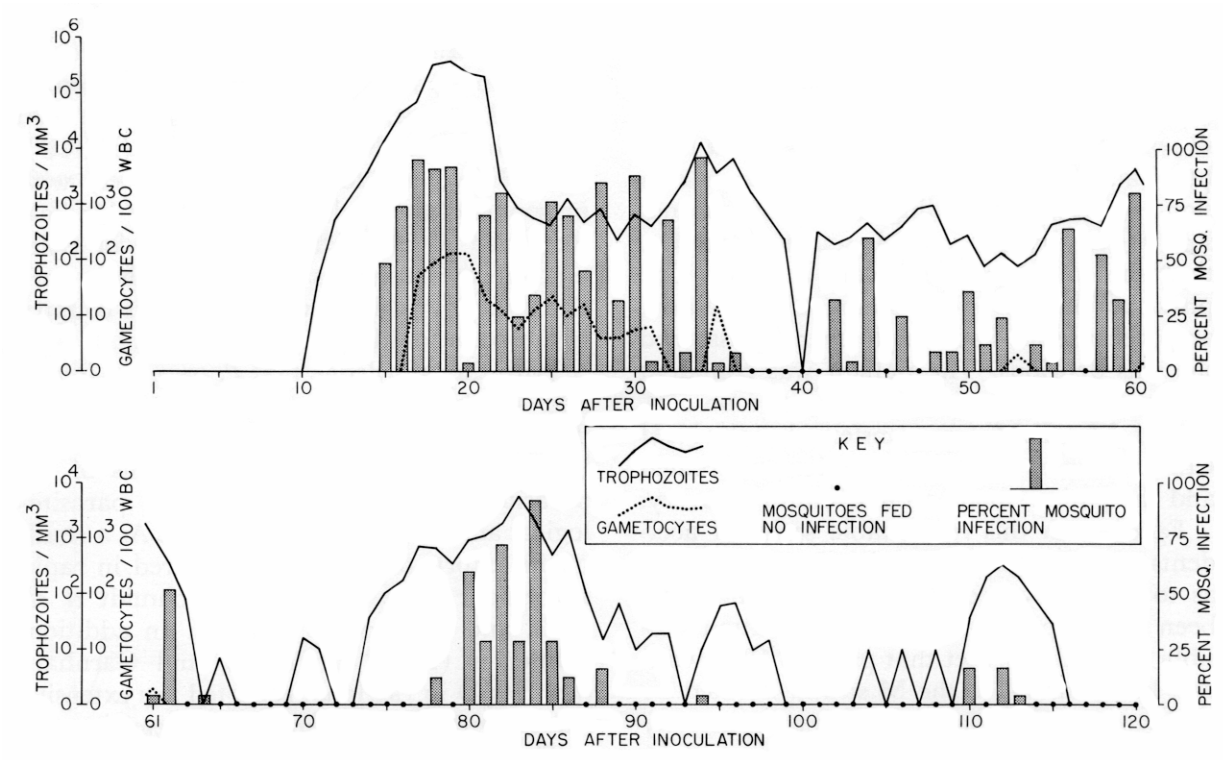

FIGURE 21.—The infectivity of *Anopheles quadrimaculatus* mosquitoes during the course of a sporozoite-induced infection with B strain *Plasmodium cynomolgi*, with its parasitemia curve, in a *Macaca mulatta* monkey.

these points, two staff members allowed mosquitoes *(A. freeborni)* infected with *P. cynomolgi* to bite them. H.A. became ill after 11 days but parasites could not be demonstrated. Blood was taken from him and injected into a clean monkey. The animal was positive for the infection 6 days later. The second man (C.S.S.), also bitten by infected *A. freeborni* mosquitoes, was positive for the infection 14 days later. It was thus proved that *P. cynomolgi* can be transmitted to man by mosquito bite. Surprisingly, almost at the same time, R.G. in Dr. Leon Schmidt's laboratory at the Christ Hospital, in Cincinnati, Ohio, came down with an accidental infection. The infecting parasite was again the B strain of *P. cynomolgi* (see Eyles *et al,* 1960; Schmidt *et al,* 1961).

After this rash of accidental infections, it was evident that mosquitoes infected with this parasite should be handled with the same respect accorded those infected with the human malarias. We did not expect further accidents, but in this we were mistaken. In mid-May, Dr. Schmidt sent mosquitoes infected with the B strain cynomolgi parasites to Dr. Clay Huff at the Naval Medical Research Institute in Bethesda, Maryland. Mrs. D.M., with wide experience in handling infected mosquitoes, took charge of them. On 4 July, she became ill with a high fever and was taken to a local hospital. On the second day following admission, when the attending physician was not sure of the diagnosis, D.M. suggested to him that she might have monkey malaria. The physician had never heard of monkey malaria, and, because she had a high fever, he assumed she was delirious. The patient was confident that she had simian malaria, a fact soon confirmed. Mrs. D.M. was transferred to the NIH Clinical Center where the infection was treated and the patient made an uneventful recovery.

Faced with this remarkable series of accidents so close together, one is moved to inquire as to why they occurred and why they had been absent previously. The explanation possibly lies with the fact that prior to 1959 work had been limited to the M strain, isolated by Mulligan in 1935, and maintained, generally by blood-inoculation, in widely scattered laboratories here and abroad. The M strain was transmitted to man with some difficulty (Coatney *et al,* 1961, and Schmidt *et al,* 1961), and when initially successful, it produced a mild disease. The B strain, however, possibly due to its recent isolation, was more readily acceptable to the human host. Also, the vector may have played an important role. *Anopheles quadrimaculatus,* the old standby in this country, is a relatively poor vector, but *A. freeborni* is highly efficient. Interestingly enough, similar accidents have been few or absent during the last 10 years, probably due to more careful handling of infected mosquitoes.

This brace of accidents, coupled with the intentional natural infections in man, indicated a zoonosis of unknown proportions, but one which might have a profound effect on the emerging world-wide program of malaria eradication, toward which the U.S. was appropriating some 60 million dollars per annum. It was considered imperative that we have information on this subject without delay. To obtain such information, studies would have to be carried out in the area where *P. cynomolgi* is endemic, namely-Malaya.

The logical one to inaugurate these studies was Dr. Eyles. When offered the chance to carry out a study of simian malaria in depth, he accepted with enthusiasm. He arrived in Kuala Lumpur, Malaya on 17 August 1960 to head the Far East Research Unit (FERU), LPC, NIH, and to work cooperatively with personnel of the Malayan Institute of Medical Research (IMR).The productivity of Eyles and his coworkers was prodigious. Their work and that of the LPC workers in this country is the basis for this monograph.

With the knowledge that *P. cynomolgi* infects and produces disease in man, it was felt desirable to embark on a more intensive study involving both the M and B strains of the parasite, with special emphasis on parasitological and clinical aspects in man. As a result of this effort, three papers appeared in rapid succession: Beye *et al,* 1961, Schmidt *et al,* 1961, and Contacos *et al,* 1962. In addition, Schneider (1961) in France, and Garnham *et al* (1962) in England, reported less extensive studies with the B strain of *P. cynomolgi.*

The results of our effort which involved some 56 patients (34 B strain, 22 M strain)

infected via mosquito bite *(A. freeborni* or *A. quadrimaculatus)* or by the inoculation of parasitized blood, can be summarized about as follows:

(1) Negroes are refractory to infection with this parasite as are some Caucasians.

(2) Monkey to man, man to man, and man to monkey transmission of the infection via mosquito bite is not only possible, but, at times, relatively easy to accomplish. The bite of a single infected mosquito resulted in a patent infection in one of 3 volunteers. The prepatent period was 19 days. The patient experienced 4 tertian fever cycles with a maximum temperature of 103° F. (Contacos and Coatney, 1963). Also, the same authors showed that the infection in a monkey (PT strain) brought directly from the field, in contrast to the long laboratory-residence of the M strain and the 3 year laboratory-residence of the B strain, could be transferred to man by mosquito bite at the first attempt. The prepatent period was 20 days.

(3) The prepatent period with either strain is about 19 days, with a range of 15 to 20 days for the B strain and 16 to 37 days, with one exception of 82 days, for the M strain.

(4) The maximum parasitemia is somewhat higher with the M strain, about 300 parasites per mm^3 as against 150 per mm^3 for the B strain. (One M strain patient, infected by blood-inoculation, developed a parasite count of 8,300 per mm^3.)

(5) There were no differences in the duration of parasitemia in the 2 strains.

(6) The first fever appeared between 16 and 19 days.

(7) The maximum temperature was 105.2° F.

(8) Tertian fever patterns were not the rule but prominent in some patients.

Clinical symptoms in patients infected with either strain consisted of cephalgia, anorexia, myalgia, and nausea, in that order. The symptoms were usually present only during febrile episodes, were of moderate severity, and easily controlled by simple medications. The most prominent physical findings were splenomegaly and hepatomegaly.

We have produced infections in many volunteers with *P. cynomolgi* and from them we have selected 29 B strain infections (13 sporozoite- and 16 blood-induced) (Fig. 22), and 26 M strain infections (11 sporozoite- and 15 blood-induced) (Fig. 23), none of which received treatment, whose parasite counts were known for the first 50 days (an arbitrary cutoff point) of their infection. Perusal of these figures will show that the parasitologic picture coincides with what was described earlier. The clinical manifestations in these patients were in the same vein.

Schneider (loc. cit.) had 3 patients, one infected by sporozoites and the other 2 by the inoculation of parasitized blood. He reported mild fever episodes, none higher than 100° F, accompanied by very low parasitemias.

The following year, Garnham *et al* (loc.

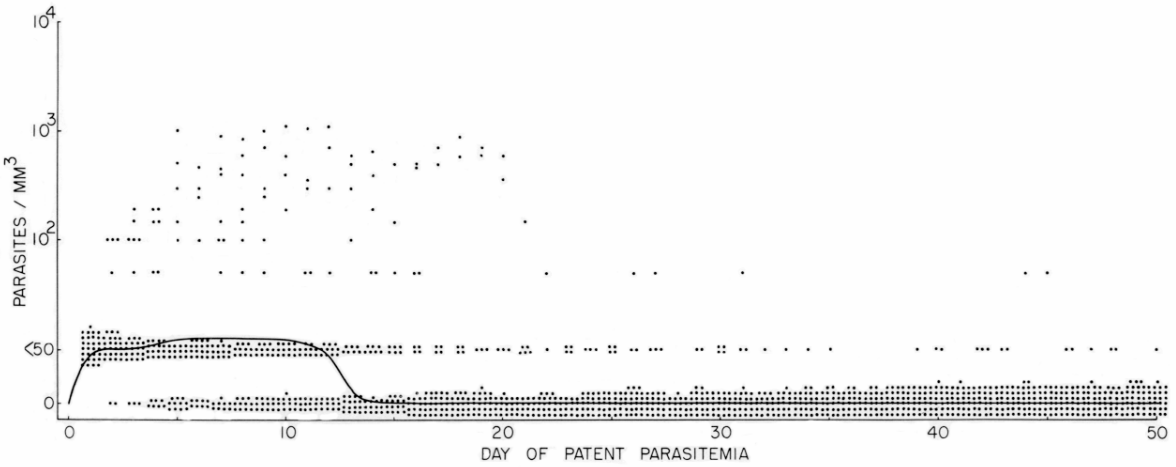

FIGURE 22.—Individual parasite counts and median parasitemia curve for 29 infections of B strain *Plasmodium cynomolgi* in man (13 sporozoite- and 16 blood-induced).

cit.) reported on sporozoite-induced infections in 14 patients (8 by mosquito bites and 6 by intravenous injections of sporozoites). The maximum temperature recorded was 104° F. The earliest prepatent period was 11 days and the average incubation period was 13 days. The infections exhibited the typical behavior of relatively severe symptoms with low parasitemias. All the infections were treated early, but there is little doubt that had they been allowed to continue, they would have exhibited the usual pattern of the untreated infection.

These studies of the early sixties convinced the most skeptical that *P. cynomolgi* was a zoonosis of unknown potential. In fact, the senior author had gone so far as to predict that *P. cynomolgi* would be the first field-acquired simian malaria in man; it was not to be (see Chapter 26).

Later attempts to infect man with *P. cynomolgi* experimentally embrace work in 2 different areas of the Orient. Dissanaike *et al* (1965) gave blood parasitized with *P. cynomolgi ceylonensis* (= our strain C) to each of 4 patients; 2 other patients were bitten by heavily infected *A. atroparvus* mosquitoes. (The primate host was not given.) None of these patients became infected. The length of the observation period was not given. In the same year, Dissanaike (1965) reported giving parasitized blood to 4 other patients; 2 received *P. cynomolgi* and *P. shortti* (= our OS strain *P. inui)* and the other 2 got *P. cynomolgi ceylonensis* (= our C strain) and *P. fragile.* None of the patients evidenced infection during an observation period of 30 days. Here, and probably in the earlier cases, too, the observation period was only 30 days. It is not unlikely that a longer period of observation would have turned up an infection in some of the recipients.

Bennett and Warren (1965) using a strain *of P. cynomolgi* isolated from an *M. irus* monkey taken in Cambodia found it infective to man via the bites of *A. maculatus* mosquitoes. The prepatent period was 21 days. In 1970, Cheong and Coombs transmitted *P. cynomolgi* to man by mosquito bite.

In our own studies we have transmitted the Gombak strain to one of 2 men exposed on one occasion, the prepatent period was 52 days; and the Smithsonian to each of two men, the prepatent periods were 25 and 26 days, respectively.

Host Specificity

Plasmodium cynomolgi naturally infects *Macaca irus* (= *fascicularis), M. nemestrina* (Eyles *et al,* 1962), *M. radiata* (Prakash and Chakrabarti, 1962), *M. cyclopis* (Inoki *et al,* 1951), *M. sinica* (Dissanaike, 1963), *M. mulatta* (Schmidt personal communication), *Presbytis cristatus* (Eyles *et al,* 1962a), and *P. entellus* (Dissanaike *et al,* 1965).

Experimentally, infections have been

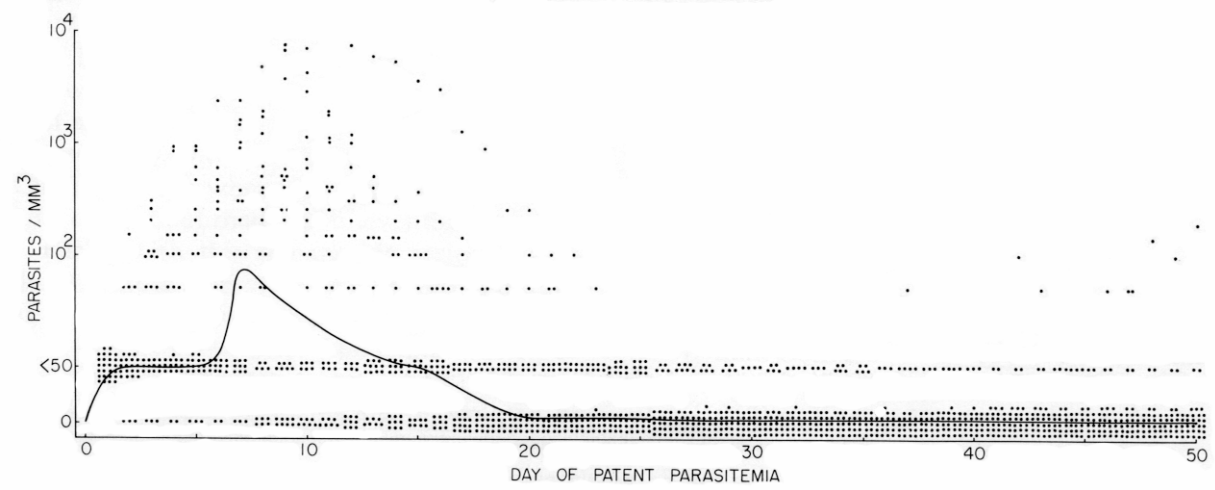

FIGURE 23.—Individual parasite counts and median parasitemia curve for 26 infections of M strain *Plasmodium cynomolgi* in man (11 sporozoite- and 15 blood-induced).

obtained in *M. mulatta, Cercopithecus aethiops* (Huff and Coulston, 1944), *Cebus capucinus* (Garnham, 1959), *Papio papio* (Garnham, 1959), and in man (Eyles *et al,* 1960; and others).

Plasmodium cynomolgi is considered to be an oligoxenous parasite since it is infective to and transmitted by a wide variety of coindigenous and exotic species of mosquitoes. Because of this, it has been used extensively in experimental studies. In Table 5, we have listed those anopheline mosquitoes which have been tested for their susceptibility to infection with this parasite. In addition, *Mansonia*

TABLE 5.—Anopheline mosquitoes tested for susceptibility to infection with *Plasmodium cynomolgi* and those shown to be vectors experimentally.

Mosquito species	Level of susceptability	Transmission	References
Anopheles annularis	High	–	Mulligan, 1935
A. annularis	Moderate		Ind. Res. Fund. As., 1947
		–	
A. aconitus	Low	–	Warren, *et al*, 1963
A. aconitus	Low		Bennett *et al*, 1966
		+	
A. albimanus	Low	–	Eyles, 1960b
A. albimanus	Low	–	Omar, 1968
A. albimanus	Low		Omar, 1968a
		–	
A. argyropus	Low		Bennett *et al*, 1966
		–	
A. aztecus	High	+	Garnham and Lainson, 1957
A. aztecus	High	–	Garnham, 1959
A. aztecus	High		Dissanaike *et al*, 1965
		–	
A. atroparvus	Refractory	–	Mayer, 1908
A. atroparvus	Unknown	–	Weyer, 1937
A. atroparvus	High	+	Rodhain and van Hoff, 1940
A. atroparvus	High	+	Hawking *et al*, 1948
A. atroparvus	High	+	Shortt and Garnham, 1948
A. atroparvus	High	+	Collins *et al*, 1965
A. atroparvus	High	–	Dissanaike *et al*, 1965
A. atroparvus	High		Omar, 1968
		+	
A.b. balabacensis	High		Collins, 1969
		+	
A.b. introlatus	Moderate	–	Bennett *et al*, 1966
A.b. introlatus	Low		Warren *et al*, 1963
		–	
A. barbirostris	High	–	Ind. Res. Fund. As., 1947
A. barbirostris	Refractory	–	Warren *et al*, 1963
A. barbirostris	Low	–	Warren and Wharton, 1963
A. barbirostris	Moderate		Bennett *et al*, 1966
		–	
A. baezai	Refractory	–	Warren *et al*, 1963
A. baezai	Refractory		Bennet *et al*, 1966
		–	
A. campestris	Moderate	–	Bennett *et al*, 1966
A. campestris	Low		Warren *et al*, 1963
		–	
A. crawfordi	Low	–	Warren *et al*, 1963
A. crawfordi	Low		Bennett *et al* 1966
		–	
A. culicifacies	High	–	Mulligan, 1935
A. culicifacies	High		Ind. Res. Fund. As., 1947

Mosquito species	Level of susceptability	Transmission	References
A. donaldi	Low	–	Warren *et al,* 1963
A. donaldi	Low	–	Bennett *et al,* 1966
A. elegans	High	+	Choudhury *et al,* 1963a
A. freeborni	High	+	Schmidt *et al,* 1948,1961,1963,1966,1970
A. freeborni	High	+	Eyles, 1960, 1960a
A. freeborni	High	+	Eyles *et al,* 1960
A. freeborni	High	+	Eyles and Coatney, 1962
A. freeborni	High	+	Beye *et al,* 1961
A. freeborni	High	+	Coatney *et al,* 1961
A. freeborni	High	+	Contacos *et al,* 1962
A. freeborni	High	+	Rossan *et al,* 1964
A. freeborni	High	+	Collins, 1969
A. fluviatilis	Moderate	–	Ramakrishnan and Mohan, 1962
A. fluviatilis	High	–	Choudhury *et al,* 1963
A. gambiae	High	–	Bray and Garnham, 1964; Garnham, 1966
A. gambiae	Moderate	–	Omar, 1968
A. hackeri	High	–	Warren *et al,* 1963
A. hodgkini	Low	–	Warren *et al,* 1963
A. hodgkini	Low	–	Bennett *et al,* 1966
A. hyrcanus	Moderate	–	Ind. Res. Fund. As., 1947
A. indiensis	Low	–	Warren *et al,* 1963
A. indiensis	Low	–	Bennett *et al,* 1966
A. kochi	High	+	Warren *et al,* 1963
A. kochi	High	+	Bennett *et al,* 1966
A. kochi	Moderate	–	Green, 1932
A. lesteri	Moderate	+	Bennett *et al,* 1966
A. letifer	Moderate	+	Warren and Wharton, 1963
A. letifer	Moderate	+	Bennett *et al,* 1966
A. leucosphyrus	High	–	Warren *et al,* 1963
A. leucosphyrus	Low	–	Bennett *et al,* 1966
A. maculatus	Low	–	Green, 1932
A. maculatus	High	–	Mulligan, 1935
A. maculatus	High	+	Warren *et al,* 1963
A. maculatus	High	+	Bennett *et al,* 1966
A. maculatus	High	+	Collins, 1969
A. peditaeniatus	Low	–	Warren *et al,* 1963
A. peditaeniatus	Low	+	Bennett *et al,* 1966
A. philippinensis	Moderate	+	Warren *et al,* 1963
A. philippinensis	Moderate	+	Bennett *et al,* 1966
A. pujutensis	Refractory	–	Warren *et al,* 1963

Mosquito species	Level of susceptability	Transmission	References
A. quadrimaculatus	High	+	Coggeshall, 1941
A. quadrimaculatus	Moderate	+	Wolfson and Winter, 1946
A. quadrimaculatus	High	+	Huff and Coulston, 1948
A. quadrimaculatus	High	+	Hawking *et al,* 1948
A. quadrimaculatus	High	+	Coulston, 1949
A. quadrimaculatus	High	+	Eyles, 1960a
A. quadrimaculatus	High	+	Beye *et al,1961*
A. quadrimaculatus	High	+	Collins *et al,* 1965
A. quadrimaculatus	High	+	Collins, 1969
A. riparis	High	–	Warren *et al,* 1963
A. riparis	Moderate	–	Bennett *et al,* 1966
A. roperi	Low	–	Bennett *et al,* 1966
A. sacharovi	High	–	Omar,1968
A. separatus	Low	–	Warren *et al,* 1963
A. separatus	Moderate	+	Bennett *et al,* 1966
A. sinensis	Low	–	Warren *et al,* 1963
A. sinensis	Low	+	Bennett *et al,* 1966
A. splendidus	High	–	Mulligan, 1935
A. stephensi	Moderate	–	Garnham and Lainson, 1957
A. stephensi	High	–	Garnham,1959
A. stephensi	Moderate	+	Ramakrishnan and Mohan, 1962
A. stephensi	High	–	Choudhury *et al,* 1963a
A. stephensi	High	+	Collins *et al,* 1965
A. stephensi	High	+	Omar,1968
A. stephensi	High	–	Omar, 1968a
A. stephensi	High	+	Collins, 1969
A. stephensi	High	–	Hawking *et al,* 1966, 1968
A. stephensi	High	–	Dissanaike *et al,* 1965
A. subpictus	Moderate	–	Ind. Res. Fund. As., 1947
A. subpictus	Refractory	–	Warren *et al,* 1963
A. subpictus	Refractory	–	Bennett *et al,* 1966
A. sundaicus	High	+	Warren *et al,* 1963
A. sundaicus	High	+	Bennett *et al,* 1966
A. tessellatus	Refractory	–	Warren *et al,* 1963
A. tessellatus	High	+	Choudhury *et al,* 1963
A. umbrosus	Low	–	Warren *et al,* 1963
A. vagus	Moderate	–	Warren *et al,* 1963
A. vagus	Low	–	Green, 1932
A. vagus	Moderate	+	Bennett *et al,* 1966

TABLE 6.--Comparative infectivity of *Plasmodium cynomolgi* to 23 species of anophelines.

Mosq. species Comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
Mac						100
Mac : Bal	30	225	190	81.8	81.1	148.7
Mac : F-1	58	405	365	80.5	83.6	116.6
Mac : St-1	28	316	269	49.4	68.8	102.8
Mac : Sun	11	102	89	92.2	75.3	100.1
Mac : Q-1	56	489	515	65.6	58.8	78.2
Mac : Koc	15	257	92	84.4	79.3	76.2
Mac : Bar	14	137	111	91.2	22.5	35.3
Mac : Les	6	59	62	84.7	54.8	27.8
Mac : Phi	10	123	70	74.0	75.7	21.5
Mac : Vag	22	263	168	83.3	46.4	17.0
Mac : Hod	3	49	9	65.3	11.0	9.9
Mac : Let	14	150	208	79.3	47.6	8.7
Mac : Sep	3	34	14	50.0	14.3	5.0
Mac : Sin	10	107	56	95.3	32.1	4.6
Mac : Atr	7	90	29	81.1	44.8	4.4
Mac : Cam	3	22	10	100.0	30.0	1.7
Mac : Ped	12	158	166	72.8	4.2	0.8
Mac : Umb	3	11	79	100.0	29.1	0.8
Mac : Arg	7	75	126	100.0	11.5	0.6
Mac : Alb	18	204	313	71.6	3.2	0.3
Mac : Don	10	107	71	83.2	12.7	0.2
Mac : Rop	2	15	12	100.0	8.3	0.1

* Mac = *A. maculatus,* Bal = *A. b. balabacensis,* F-1 = *A. freeborni,* St-1 = *A. stephensi,* Sun = *A. sundaicus,* Q-1 = *A. quadrimaculatus,* Koc = *A. kochi,* Bar = *A. barbirostris,* Les = *A. lesteri,* Phi = *A. philippinensis,* Vag = *A. vagus,* Hod = *A. hodgkini,* Let = *A. letifer,* Sep = *A. separatus,* Sin = *A. sinensis,* Atr = *A. atroparvus,* Cam = *A. campestris,* Ped = *A.peditaeniatus,* Umb = *A. umbrosus,* Arg = *A. argyropus,* Alb = *A. albimanus,* Don = *A. donaldi,* Rop = *A. roperi.*

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

uniformis has been experimentally infected with several strains of *P. cynomolgi* (Warren *et al,* 1962). Both oocyst and sporozoite infections were demonstrated but no transmissions were obtained (Warren and Wharton, 1963; Bennett *et al,* 1966). *Culex vishnui* (Mulligan, 1935), C. *tritaeniorhynchus* (Warren and Wharton, 1963) and *Aedes butleri* (Warren and Wharton, 1963; Bennett *et al,* 1966) have been reported susceptible to infection as far as the oocyst stage only.

In our own studies, 23 species of anophelines have been compared for susceptibility to infection with *P. cynomolgi* (Table 6). The most readily infected was *A. b. balabacensis* and the least susceptible was *A. roperi.*

Immunity and Antigenic Relationships

Mulligan and Sinton (1933) demonstrated that immunity produced by a given strain of the parasite in monkeys appears to be specific mainly for the same strain. There was some evidence, however, to suggest a slight degree of common immunity. Chronic infections with *P. cynomolgi* conferred effective immunity against the clinical effects of superinfection with the same strain of parasite. They were unable, however, to demonstrate any cross-immunity between infections due to *P. knowlesi* and *P. cynomolgi.* Singh and Singh (1940) found that

chronic infections due to *P. cynomolgi* and *P. inui* failed to prevent, or modify, the course of infection with heterologous parasites. It was also shown that *P. cynomolgi* produces an immunity to homologous superinfection which remains effective for at least 18 months.

In 1966, Voller *et al* reported on cross-immunity studies with a number of species of monkey malaria. They demonstrated that infections with *P. cynomolgi bastianellii* and *P. cynomolgi ceylonensis* produced no cross-immunity either to each other or to *P. knowlesi, P. coatneyi, P. fragile, P. inui, P. inui shortti,* and *P. gonderi.* In a later extensive immunological study, Voller and Rossan (1969, 1969a, 1969b) demonstrated that *M. mulatta* monkeys with chronic infections due to *P. knowlesi* were still susceptible to infection with *P. cynomolgi bastianellii.* Animals with chronic infections with *P. cynomolgi bastianellii* were immune to challenge with the homologous parasite and to a high degree to *P. cynomolgi ceylonensis.* In contrast, monkeys with chronic infections of *P. cynomolgi bastianellii* produced severe infections when challenged with *P. cynomolgi ceylonensis.* Their studies also indicated that parasite populations isolated from a late relapse of *P. cynomolgi* were immunologically different from those isolated from the primary infection and from an early relapse. They concluded that these variants arose from different antigenic variants released from the liver.

The first observations on the serologic cross reactions between *P. cynomolgi* and *P. vivax* were made, using the fluorescent antibody test, by Tobie and Coatney (1961) and Tobie *et al* (1962). They found that when antisera to *P. cynomolgi* from human volunteers was allowed to react with the homologous and heterologous parasites, considerable cross reaction was obtained. They noted, however, that the maximum antibody titers were obtained with the homologous parasite. Voller (1962) demonstrated a strong IFA cross reaction between *P. bastianellii* (= *P. cynomolgi bastianellii)* and *P. vivax, P. gonderi,* and *P. osmaniae* (= *P. inui shortti)*. Subsequent studies indicated that antisera to *P. falciparum, P. malariae,* and *P. ovale* would also cross react with the *P. cynomolgi* antigen (Kuvin and Voller, 1963; Collins *et al,* 1966a; Meuwissen, 1968).

In our studies (Collins *et al,* 1966), antisera to *P. cynomolgi* gave a fluorescent antibody cross reaction at a high level to *P. fieldi* antigen (mean reciprocal titer ratio of 100:76) and lesser reactions to *P. knowlesi, P. gonderi,* and *P. brasilianum* (mean reciprocal titer ratios of 100:54, 100:36, and 100:31, respectively). In the reverse procedure, *P. cynomolgi* antigen gave the highest cross reaction to *P. inui, P. knowlesi, P. fragile,* and *P. fieldi* (mean reciprocal titer ratios of 100:46, 100:41, 100:33, and 100:31, respectively).

El-Nahal (1967), using the exoerythrocytic stages of *P. cynomolgi* as antigen in a fluorescent antibody test, showed that whereas the homologous antisera responded well, the heterologous antisera to *P. inui* and *P. malariae* failed to respond.

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(NS) = Not seen.