

Plasmodium falciparum (Welch, 1897)

SYNONYMS: *Oscillaria malariae* Laveran, 1881; *Plasmodium malariae* Marchiafava and Celli, 1885; *Laverania malariae* Feletti and Grassi, 1890; *Haemamoeba praecox* Grassi and Feletti, 1890, *partim*; *Ematozoo falciforme* Antolisei and Angelini, 1890; *Haemamoeba immaculata* Grassi, 1891; *Haemamoeba laverani* Labbe, 1894; *Haematozoon falciforme* Thayer and Hewetson, 1895; *Haematozoon falciparum* Welch, 1897; *Haemosporidium sedecimanae* Lewkowicz, 1897; *Haemosporidium undecimanae* Lewkowicz, 1897; *Haemosporidium vigesimotertiana* Lewkowicz, 1897.

As mentioned in an earlier chapter, Laveran in his early studies (1880, 1881) of the parasites of human malaria saw each of the principal species and in 1881 used the name *Oscillaria malariae* for these parasites, including the crescent-shaped bodies; then, and later, he steadfastly held to the belief that only one species was involved. Garnham (1966) gives an interesting account of Laveran's first observations of the falciparum parasite in 1880 in the blood of a young soldier who had been in Algeria for about a year. In 1892, Grassi and Feletti, as an honor to Laveran, proposed the genus name *Laverania* which was zoologically correct, providing two genera are recognized. However, since most authors recognize only the genus *Plasmodium* Marchiafava and Celli, 1885, the latter took precedence under Opinion No. 104 of the International Commission on Zoological Nomenclature, 1928.

The confusion which surrounded the naming of this parasite was linked to its masked periodicity and to the presence of crescent-shaped bodies. Marchiafava and Bignami (1892)

resolved some of the mysteries of the parasite, as seen in the peripheral blood, by setting forth its 48-hour cycle, and showing that the small parasites and the crescents were parts of the same cycle. On clinical grounds, they pointed out its perniciousness. Mannaberg (1893), in his concisely written little book, pointed out the error of ascribing triple etiology, as Feletti and Grassi (1890) had done, to the malignant tertian parasite and his illustrations, colored Plate IV, leave little doubt that he was familiar with the circulating blood forms of the parasite.

Several different names were proposed for the parasite between 1885 and Welch's *Haematozoon falciparum* of 1897. The latter name called attention to the sickle-shaped parasites and, probably for that reason, was widely accepted by the scientific community, even though taxonomically incorrect. Such a situation is intolerable because of the priority rule and a long struggle began among taxonomists to resolve the dilemma.

In 1929, Sergent *et al* made an exhaustive study of the situation and came to the conclusion that the correct name for the malignant tertian parasite should be *Plasmodium praecox* Grassi and Feletti, 1890. In 1935, Giovannola reexamined the problem and decided that the correct name should be *Plasmodium immaculatum* (Grassi and Feletti, 1892); but, in 1938, Christophers and Sinton pointed out that if that name were accepted, it must be credited to Grassi, 1891. The latter authors went on to point out that *malariae* was the name applied originally by Laveran and is, therefore, the *de jure* name.

It was abundantly clear that strict adherence to the rules of zoological nomenclature would create intolerable confusion and when Sergent *et*

al (1939) withdrew their proposal of 1929, there appeared a united front, joined by Coatney and Young in 1941, for the general adoption of the commonly used *de facto* name. However, a ruling was necessary to give status to the consensus. At the 1954 meeting of the International Commission on Zoological Nomenclature, the trivial name *falciparum* of Welch (1897) was validated but privilege was given for its use with either the genus *Plasmodium* or with *Laverania* (see Hemming, 1954). The ruling settled the specific name, and, it was hoped, at least in some quarters, that in spite of privilege, *Plasmodium* would be the accepted name for the genus. However, Bray (1958) redefined the genus *Laverania* and consigned two species to it: *falciparum* and *reichenowi*. Although he, and certain others, felt strongly about the designation, he was willing to concede "the use of the genus is not obligatory." There was a great deal of heated discussion regarding Bray's proposal, most of it without the benefit of printers ink, with the result that, in 1963, he relegated *Laverania* to subgeneric rank. During the years since 1963, the use of *Laverania* as a genus name has continued to lose favor. In fact, Garnham (1966), although he supported Bray's proposal in 1958, fails to mention its revival after the 1954 decision. We feel, that in the interest of uniformity and convenience, the name of the malignant tertian parasite should be *Plasmodium falciparum* (Welch, 1897).

Plasmodium falciparum has a worldwide distribution and is concentrated in the tropics and subtropics. It invades the temperate zone and, as a consequence, it used to be common in southeastern United States, the littoral areas of the Mediterranean, and in the Balkans. It has since disappeared from those areas generally as a result of better economic conditions, good control, and/or eradication programs.

Altitude has an important bearing on the transmission of *P. falciparum*, and other species, too, but the height at which it disappears is variable depending on the temperature at which the vector can maintain itself. Under ordinary circumstances, transmission fails above 1500 meters. Exceptions are not uncommon, however.

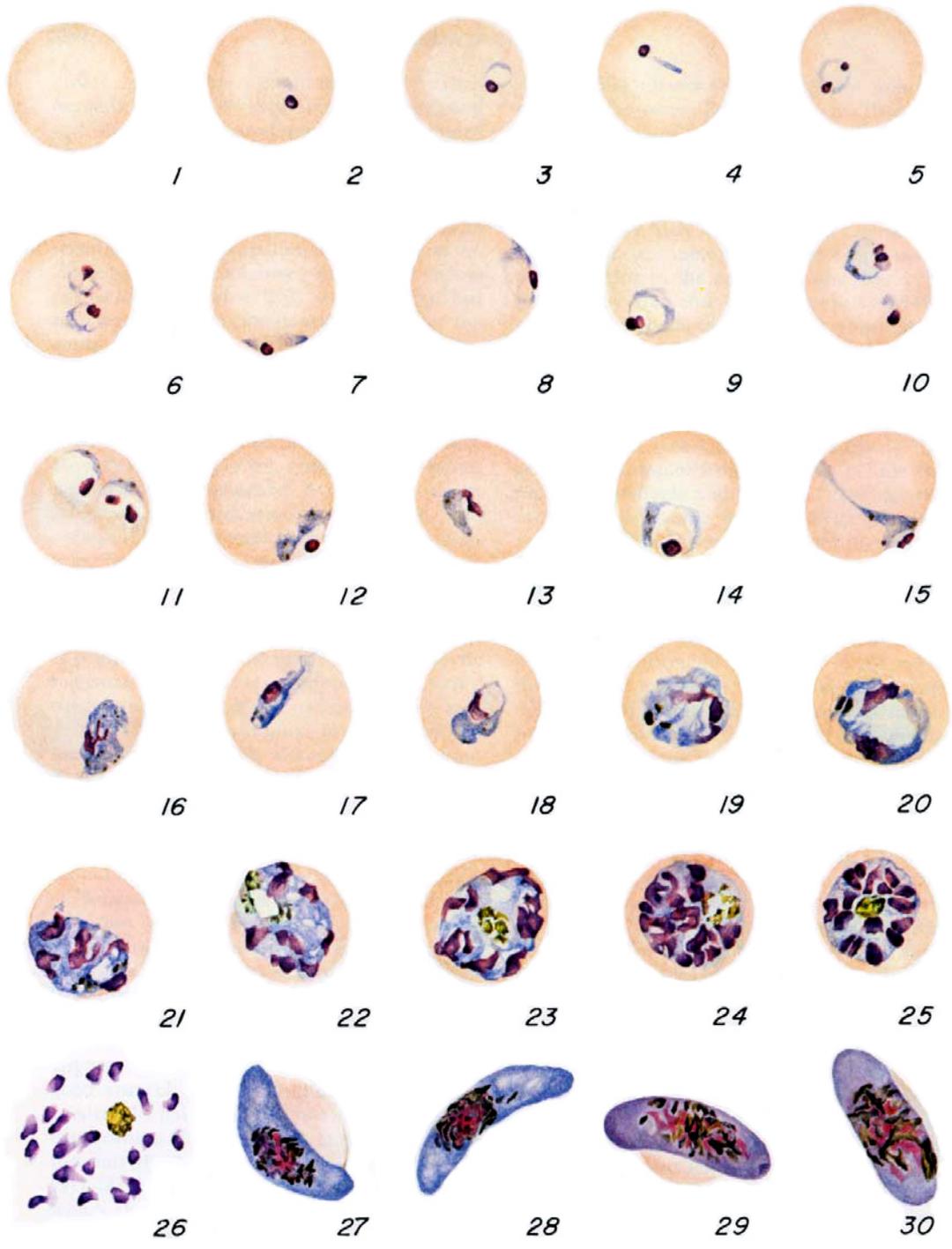
Polumordvinov (1945) reported infections in southern Tadjikistan at 2750 and 2850 meters which is the limit of human settlements in that region. Garnham (1948) described an epidemic in the highlands of Kenya, as high as 2600 meters, where at Kericho, for example, the parasite rate was 8 percent prior to the epidemic, but rose to 36 percent by the end of it. Hackett (1945), working in Bolivia, demonstrated transmission at 2600 meters.

There are many accounts of havoc among early civilizations which is attributed to malignant malaria, but proof that malaria was the actual culprit was lacking until early in this century. Examples from this era include the report by Raffaele and Coluzzi (1949) for the area around Cassino in Italy. Prior to 1943, malaria, although present, was of little importance. In 1945, following the bloody fighting between the German and the Allied Armies, with destruction of dykes and other control measures in 1943-44, about 100 percent of the people were infected; 43 percent of the infections were *P. falciparum*. The mortality in some villages of the area was 10 percent. In this hemisphere, the classic example of introducing an efficient vector into a susceptible *P. falciparum* population is that of *Anopheles gambiae* in northeast Brazil as recounted by Soper and Wilson (1943). The first *A. gambiae* probably arrived in the area from Africa in 1929; its transmission potential was recognized in 1930, but scant attention was paid to it until it reached the Assu and Apode valleys in Rio Grande do Norte in 1938. It spread over some 12,000 square miles leaving illness, death, and desolation until finally eradicated in 1940 by an encircling technique which, at its inception, many malariologists called an "audacious experiment."

Among the human malarias, *P. falciparum* is considered the youngest evolutionarily and the least efficient as a parasite because its malignant nature tends to eliminate its host.

We have studied many different strains of *P. falciparum*, some of which will be discussed later in this chapter.

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PLASMODIUM FALCIPARUM

J. H. Nicholson

Cycle in the Blood

PLATE XLII

The youngest ring forms of *Plasmodium falciparum* are smaller than those of other human malarias and are commonly referred to as tiny, hair-like rings, with a vacuole, and a prominent nucleus. Sometimes, there is an accessory chromatin dot (Fig. 2-5). Multiple invasion of the host cell by equal-aged parasites is more common in this species than in the other human malarias (Figs. 6, 10, 11). Field and Shute (1956) illustrate 7 ring-stage parasites in a single cell and state that "eight rings in a cell has been recorded." Appliqué or accollé forms are common and, hence, have some diagnostic value. As development proceeds, the overall size of the parasite is increased, the vacuole and the nucleus of the parasite become more prominent (Fig. 14), and tenue forms (Fig. 15) may appear; aside from these abnormal forms (see Field and Shute, 1956), there is no appreciable amoeboidity. The parasite now becomes smaller and more compact, the cytoplasm stains a deep blue, it loses its vacuole, the nucleus ceases to be circular, and dark pigment grains appear in the cytoplasm (Figs. 16-19).

At this juncture, the number of parasites in the peripheral blood decreases due to their penchant for retreating into the deeper circulation—a practice common to *P. coatneyi*, too—so that in cases of high synchronicity, it is sometimes difficult to find late developing forms. In general, however, the phenomenon of asynchronicity produces enough tardy forms to permit following the remainder of the cycle. As a rule, the presence of appreciable numbers of segmenters in the peripheral blood is an indicator of grave consequences, but this is not always the case. In our own experience, we have seen a case in which the patient was not particularly ill, was ambulatory, and had a

parasite count of about 5,000 per mm³, yet he continued to show a high proportion of segmenters for several days.

During schizogony, the nucleus divides repeatedly, the parasite increases in size until it may occupy a large part of the host cell. At first the pigment comes together in small aggregates, but, as the parasite nears maturity, it collects in a single yellowish-brown mass. The mature schizont is less symmetrical than those of other human malarias and its merozoites number 8 to 20; the usual number is about 16 (Figs. 20-25).

One of the striking features of erythrocytes infected with the asexual parasites is the early development of Maurer's dots, or clefts, which make their appearance shortly after the hair-like ring stage. As the development of the parasite proceeds, these abnormalities become more pronounced. They are demonstrable in the parasitized red cells only under certain staining procedures, not ordinarily applied, and therefore are not shown in our plate.

The mature gametocytes are unique among human malarias because of their sickle or crescent shape, a feature well appreciated by Laveran. In most malarias, the gametocytes appear about the same time as the asexual forms, but in falciparum malaria, it is about 10 days after the first appearance of the asexual forms that they appear as a wave of full grown parasites. Preceding their appearance, the young gametocytes have been growing in the blood spaces of the spleen and bone marrow.

The macrogametocyte is relatively slender, has pointed ends, and is generally longer than the microgametocyte. The cytoplasm stains a decided blue. The nucleus is compact and may be masked by pigment granules which appear to cover it. The red cell may be seen as stretching across the curvature of the gametocyte (Figs. 27,

PLATE XLII.—*Plasmodium falciparum*.

Fig. 1. Normal red cell.

Figs. 2-11. Young trophozoites.

Figs. 12-15. Growing trophozoites.

Figs. 16-18. Mature trophozoites.

Figs. 19-22. Developing schizonts.

Figs. 23-26. Nearly mature and mature schizonts.

Figs. 27, 28. Mature macrogametocytes.

Figs. 29, 30. Mature microgametocytes.

28). The microgametocyte is sausage-shaped with blunt-rounded ends. The cytoplasm stains light blue to purplish-blue. The nucleus occupies about half the total length of the parasite. It is diffuse, and generally shows some dark red dots scattered in a pale pink area. Lying well within the periphery of the nuclear area are clustered dark brown to black pigment granules. The host cell generally hugs the body of the parasite, but may show as a bulb-like area in the slight curvature of the gametocyte (Figs. 29, 30).

The asexual cycle is 48 hours.

Sporogonic Cycle

PLATE XLIII

There have been many studies on the sporogonic cycle of *P. falciparum* since Ross (1897) described finding oocysts on the gut of a mosquito which had fed on a gametocyte carrier. Bastianelli *et al* (1898) observed pigmented oocysts in anopheline mosquitoes which had fed on an individual infected with *P. falciparum*,

and, in the same year, Grassi *et al* (1898) observed complete development of *P. falciparum* in *Anopheles claviger* (= *A. maculipennis*). In 1899, Bastianelli and Bignami not only described the development of the parasite in mosquitoes, but, also, demonstrated its transmission to man.

Shute and Maryon (1952) observed the development of oocysts of *P. falciparum* in *A. atroparvus* mosquitoes incubated at a temperature of 25° C. The black pigment granules (between 10 and 20 in number) were usually arranged (between days 3 and 7) in a double semicircle around the periphery of the oocyst. By the 8th day, the pigment was obscure; the oocysts measured from 8 to 60 μ in diameter. The sporogonic cycle was completed in 11 to 12 days. From the 3rd to the 5th day, the daily increase in oocyst diameter was approximately 4 μ . From the 6th to the 10th day, the daily increase was about 10 μ .

Our studies of the sporogonic cycle of this

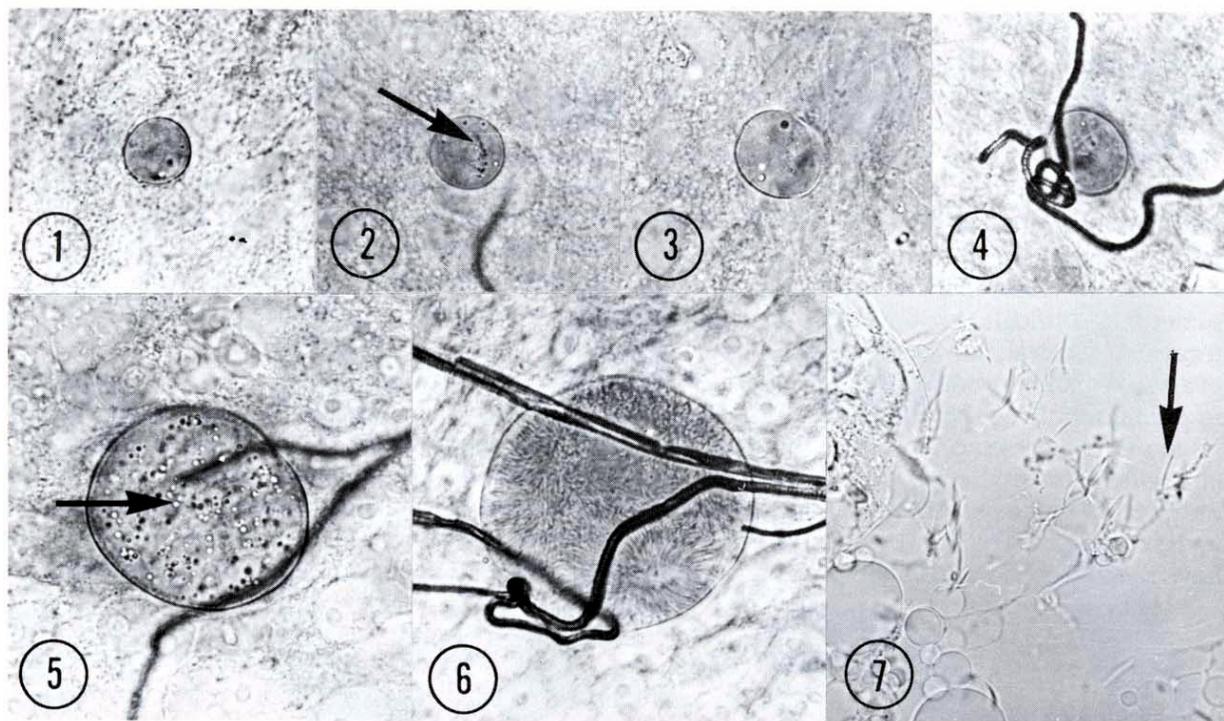


PLATE XLIII.—Developing oocysts and sporozoites of *Plasmodium falciparum* in *Anopheles freeborni* mosquitoes. X 580.

Fig. 1. 7-day oocyst.

Fig. 2. 7-day oocyst showing line of pigment.

Fig. 3. 8-day oocyst.

Fig. 4. 10-day oocyst.

Fig. 5. 14-day oocyst showing numerous small vacuoles.

Fig. 6. 14-day differentiated oocyst.

Fig. 7. Sporozoites present near salivary gland tissue 14 days after feeding.

parasite have been limited, but we have followed its development in *A. freeborni* infected with the Malayan IV and the McLendon strains of *P. falciparum*, and in *A. quadrimaculatus* infected with the McLendon strain only (Table 34). In *A. freeborni*, with the Malayan IV strain, on day 5, oocysts had mean diameters of 12 μ, with a range of 8 to 15 μ; on day 12, the mean size was 50 μ, with a range of 21 to 78 μ. There are some differences in the development of the 2 strains in *A. freeborni*. The Malayan IV had slightly larger mean oocyst diameters, but, more significantly, sporozoites were present in the salivary glands on day 12 whereas the McLendon strain required 14 days. The development in the *A. quadrimaculatus* was similar to that seen in *A. freeborni* infected with the McLendon strain. Sporozoites were present in the salivary glands on day 14.

A comparison of the oocyst growth rate of the Malayan IV strain of *P. falciparum* with that of *P. cynomolgi* (Fig. 57) shows a marked difference between the 2 parasites. The *P. cynomolgi* was much larger both with regard to mean and maximum oocyst diameters. Sporozoites were present in the salivary glands of the mosquitoes infected with *P. cynomolgi* one day sooner than in those infected with *P. falciparum*.

Experimentally, *P. falciparum* has been transmitted to man via the bites of many species of mosquitoes on numerous occasions. In that connection, Garnham (1966) lists 66 species of anophelines which will serve as hosts of *P. falciparum*.

Cycle in the Tissue

The tissue stages of *Plasmodium falciparum* have been demonstrated in experimental infections of man as well as chimpanzees. This species of human malaria differs from *P. vivax* and *P. ovale* in that the exoerythrocytic cycle is restricted to a single generation; in other words, there is no secondary exoerythrocytic or other continuing fixed tissue stage.

The tissue cycle of *Plasmodium falciparum* was first demonstrated by Shortt *et al* (1949, 1951) in liver biopsy material from a human volunteer who had been bitten by 770 Anopheles mosquitoes (93 percent infection rate) over a period of 3 days. The strain of falciparum malaria used by these authors was of Roumanian origin. The liver biopsy was taken 5% days after mosquitoes had first bitten the volunteer. The exoerythrocytic schizonts described by these authors were considered to be 4-, 5-, and 6-day stages. The 4-day schizonts were described as

TABLE 34.—Oocyst diameters of *Plasmodium falciparum* (Malayan IV and McLendon strains) in *Anopheles freeborni* and *A. quadrimaculatus* mosquitoes.

Days after Infection	Malayan IV strain			McLendon strain					
	<i>A. freeborni</i>			<i>A. freeborni</i>			<i>A. quadrimaculatus</i>		
	No.	Range	Mean*	No.	Range	Mean	No.	Range	Mean
4				118	5-11	10			
5	106	8-15	12	193	7-14	10	5	8-14	11
6	122	9-21	15	115	11-20	15	116	8-20	14
7	163	9-28	19	152	8-20	15	134	12-23	18
8	255	12-38	23	120	11-31	20	187	11-32	19
9	199	14-45	32	169	12-41	24	101	18-38	28
10	111	26-64	41	150	17-53	35	168	18-50	33
11	112	20-73	45†	132	19-61	40	126	20-58	40
12	154	21-78	50†**	150	20-72	47†	110	18-60	42
13	36	31-71	56†**	139	19-70	49†	132	27-68	48†
14				164	20-67	45†**	109	30-68	54†**
Totals	1258	8-78		1602	5-72		1188	8-68	

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

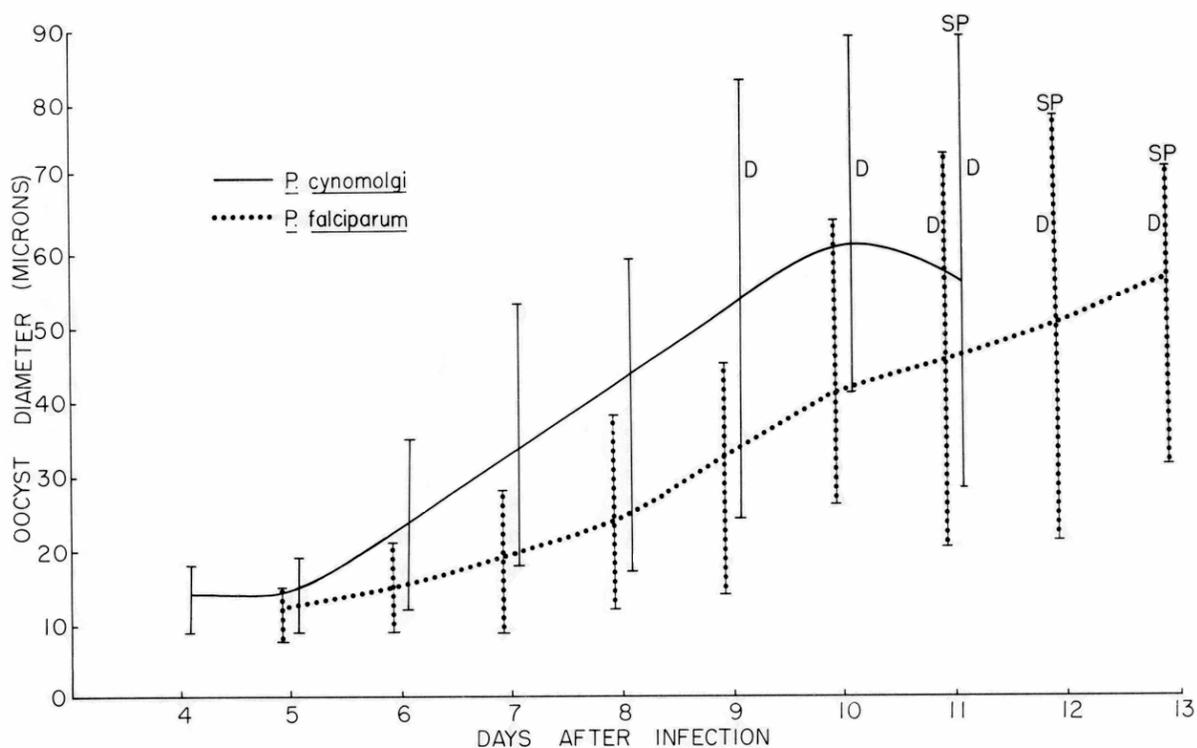


FIGURE 57.—Mean oocyst diameter curves and ranges in oocyst diameter of *Plasmodium cynomolgi* and *P. falciparum* (Malayan IV strain) in *Anopheles freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

ovoid or spherical in shape, with some tendency toward the production of lobose projections. They measured about 30 μ in diameter and were surrounded by a thin membrane. The cytoplasm was fairly dense and there were no vacuoles. Nuclei were rather sparsely distributed, slightly irregular masses measuring approximately 1.5 μ in diameter. Cytoplasm tended to condense around each nucleus; and, according to the authors, this possibly represented the first step in the formation of the so-called pseudocytomeres.

Five-day old schizonts measured roughly 50 μ in their longest dimension. The tendency to produce the lobose character was more pronounced. There was no local tissue reaction. There was a tendency for the cytoplasm to separate into areas resembling cytomeres. These pseudocytomeres varied in shape from spherical to elongate; each contained a large number of nuclei. The size of the individual nuclei, in active division, was roughly 0.8 μ . The membrane surrounding the parasites was less apparent.

The 6-day stages showed the parasite had undergone very rapid growth, expanding in an irregular fashion in many directions. Six-day forms measured 50 to 60 μ in length and were described as being more "misshapen" than *P. vivax* because of the pronounced tendency to lobosity. The number of nuclei had increased tremendously, the cytoplasm being thickly strewn with them. Near maturity, the parasite appeared to break up into small islands of cytoplasm, measuring approximately 2 μ in diameter, each island contained two very small nuclei.

With a final division of the nuclei, one has a mature schizont, measuring 60 μ or more in its greatest dimension, containing an enormous number of merozoites. Each merozoite, about 0.7 μ in diameter, consists of a nucleus with a trace of cytoplasm. The number of merozoites in a large mature schizont was estimated as roughly 40,000.

Jeffery *et al.* (1952) carried out a study, involving 14 patients, designed to demonstrate

the exoerythrocytic stages of *Plasmodium falciparum* in the human liver. Inoculation was either by the bites of heavily infected mosquitoes, the intravenous inoculation of dissected salivary glands, or both. Each volunteer received from 2 to 6 inoculations. Biopsies were performed 3 to 8 days after the first inoculation. Material from 13 of the 14 patients was negative. The 14th patient, who received a total of 8,516 mosquito bites along with the intravenous inoculation of 1,403 pairs of salivary glands, yielded material positive for falciparum tissue schizonts. The authors found 125 parasites; 100 were complete.

The smallest parasites were 15 μ in their greatest diameter. However, the authors stated that due to shrinkage, the living parasites were undoubtedly larger than 15 μ . The biopsy was done on day 6 and inoculations occurred on day 0, 1, 2, and 3, and consequently, the tissue stages could have been anything from 3 to 6 days old. The cytoplasm was described as being granular, sometimes containing small vacuoles. The outline of the parasite appeared to be wavy which was probably due to shrinkage. The smallest number of nuclei observed was about 40.

Parasites approximately 25 μ in their greatest diameter were also observed. The cytoplasm was homogeneous, contained a few vacuoles, and a larger number of nuclei. The largest parasite, about 40 μ , was considered to be nearly mature. The cytoplasm was vacuolated, the outline of the parasite was smooth, and, occasionally, small lobes were observed. The stage considered nearest maturity was described as having an increase in the complexity of the vacuoles and an apparent formation of cords and islands of cytoplasm (pseudocytomeres) from which the merozoites arise. Most parasites at this stage were 55 to 60 μ in their greatest diameter.

The parasites were found in the parenchymal tissue of the liver lying within the liver cords. The host cells were enlarged but no changes were observed in the host cell nuclei. There were no detectable morphologic reactions to the presence of the parasite. In addition, there was no leukocytic infiltration into areas around the parasite.

The parasites described by Jeffery *et al* (1952) were considered similar to those described by Shortt *et al* in 1951. However, a few differences, which may be due to interpretation, were noted. For example, very little, if any, compression of host tissues surrounding the parasite was observed by these authors. Shortt *et al* described the tendency toward lobation in the parasite. Jeffery *et al* noted the same thing, but in addition, reported that it was not unusual to find parasites, including mature schizonts, with smooth outlines, although in many others, the lobose character was extremely pronounced. Jeffery *et al* felt it was not possible to designate a particular parasite as being of a specific age since inoculations were carried out over a period of 4 days which was some 3 to 6 days before biopsy was taken. Shortt *et al* (1951) also had inoculated their patient on 3 consecutive days and correlated their parasites with time, designating them as being 4, 5, and 6 days of age on the basis of size. On this basis, then, the smallest parasite found by Jeffery *et al*, which was 15 μ , could have been 3 days old, whereas the smallest parasites found by Shortt *et al* were about twice that size and were designated as being 4 days old.

Shortt *et al* (1951), in comparing the pre-erythrocytic stages described for cynomolgi and vivax malaria with those of falciparum malaria, indicated several points of difference; namely, 1) the larger size of the mature schizont, 2) the smaller size of the merozoites, 3) the greater number of merozoites, and 4) the rapidity of development of the tissue stages to full maturity, i.e., 6 days for *P. falciparum* compared with 8 or more for *P. vivax* and *P. cynomolgi*.

Bray (1958, 1960) and Bray and Gunders (1962, 1963) studied the tissue stages of *P. falciparum* in chimpanzees. The youngest forms were 2-day stages. These were described as lying within a vacuole in the liver parenchymal cell and measuring about 4 μ in diameter. The 2-day parasite was enclosed in a well-defined envelope and contained 2 nuclei which were triangular in shape. At 2 days and 15 hours, the tissue stages measured 7 μ and contained up to 20 nuclei. Three-day forms, still within a vacuole in the liver cell cytoplasm, averaged 9 μ

and contained 25 nuclei. At 4 days, the parasite measured 18 μ and contained flocculated cytoplasm studded with about 145 large nuclei.

Bray (1958) considered the 5-day forms in the chimpanzee liver similar in all respects to the 4-day stages described by Shortt *et al* and the smaller schizont described by Jeffery *et al*. The average greatest dimension was 32 μ , with a range of 41 to 28 μ . The outline of the parasite was generally regular without lobations. The cytoplasm was rarely vacuolated. However, when vacuoles were present, they were small and rarely numbered more than 2. The contour of the parasite was indented by the host cell nucleus.

Three different types of nuclei could be seen in the chimpanzee material: 1) parasites with tiny dots of chromatin measuring 0.5 μ in diameter and usually associated with a smooth homogeneous appearance of the cytoplasm, 2) parasites with round pieces of chromatin approximately 1 μ in diameter, and 3) parasites with relatively large pieces of chromatin measuring 1.0 to 1.7 μ which were circular, oblong, or even square in shape, and these were associated with a cytoplasm which tended to display aggregates of basophilic material.

The exoerythrocytic cycle of falciparum malaria appears to be slightly longer in the chimpanzee than it is in man. Bray (1958) considered the 8-day parasites in his chimpanzee material to be nearly mature and resembled the 6-day schizonts of Shortt *et al* and the large schizonts of Jeffery *et al*.

The final stages of schizogony were described as very complex, and defined by Bray (1960) as aposchizogony. Garnham (1966) stated that the common appearance of the tissue schizont on the 6th day is that of an ovoid structure, about 40 μ in its greatest dimension, containing hundreds of cytomeres, each cytomere measuring 3 to 5 μ in diameter. The cytomeres are considered as hollow spheres, the surface studded with small nuclei. At first there are only a few of these cytoplasmic islands or cytomeres, but by the process of aposchizogony (Bray, 1960) a large number of cytomeres are eventually produced, each becoming smaller and with progressively fewer nuclei (i.e., 8, 4, 2 nuclei) with each division. At maturity,

merozoites become differentiated from the 2 nucleate cytomeres.

There is direct and indirect evidence to suggest that the exoerythrocytic cycle of falciparum malaria is limited to a single generation--the first. In other words, no true relapse occurs because no tissue stages remain after the development of the primary falciparum malaria infection. Bray (1958) found no tissue schizonts in biopsies of liver taken 12 days after infection. This contrasts greatly with the ease with which he found *P. vivax* and *P. ovale*, under similar experimental conditions, in liver biopsies from chimpanzees; *P. vivax* and *P. ovale* are relapsing malarias.

Sodeman *et al* (1969) described 6- and 7-day stages of *P. falciparum* in the liver tissue of the owl monkey, *Aotus trivirgatus*. The parasites resembled the 2.5 to 5-day stages observed in the chimpanzee and in man. The owl monkey EE bodies were considered non-viable because the host failed to develop a patent infection.

Fairley *et al* (1947), by subinoculating a large volume of blood from volunteers exposed to falciparum infection by bites of 7 to 20 infected mosquitoes, concluded that the tissue cycle ended at 6 ½ days. When blood taken 160 hours, or later, after exposure to infection, was subinoculated into volunteers, the subinoculees developed patent falciparum infections. As stated earlier, Shortt *et al* found that, blood taken from their one volunteer at 135 hours after exposure to infection with approximately 716 infected mosquitoes was infectious when inoculated into another volunteer. It appears obvious that the difference of 25 hours was probably due to the difference in the number of infective bites (maximum of 20 versus approximately 700). Ciucu *et al* (1937) showed that the tissue cycle of a Roumanian strain of falciparum malaria was completed during the 6th day after exposure to infection.

Garnham (1966) states that the prepatent period for falciparum malaria is 5½ days since the tissue schizont ruptures at that time releasing merozoites into the blood stream as indicated by experiments similar to those of Fairley, i.e., that a large volume of blood taken from the volunteer at 135 hours after exposure was injected into another volunteer and the subinoculee developed malaria.

Course of Infection

The course of infection is inaugurated with the entry of merozoites into red cells of the circulating blood, and according to Garnham (1966), Shute demonstrated a parasite in a thick blood film from a patient who had received 500,000 sporozoites of a Roumanian strain of *Plasmodium falciparum* intravenously 5 days earlier. Shortt *et al* (1951) showed that the prepatent period could be as early as 5½ days when exposure to infection was massive and blood was subinoculated into volunteers. We prefer to define prepatent period as the interval from the time of exposure to the demonstration of parasites in the blood of the host by more conventional methods; namely, the thick blood film. On this basis, the prepatent period observed by Shortt *et al*, in the volunteer from whom liver biopsy material was obtained, was 7 days. In addition, Shortt *et al* observed a prepatent and incubation period of 8 days in a patient who served as a control for mosquito infectivity and who was bitten by a total of 370 mosquitoes on 4 consecutive days.

Ciucu *et al* (1937a), in their description of 12 falciparum malaria infections, induced by the bites of infected mosquitoes and/or the intravenous inoculation of sporozoite suspensions, reported prepatent periods ranging from 11 to 20 days and incubation periods from 11 to 21 days, with medians of 12 days.

Burgess and Young (1946) experimentally transmitted the McLendon strain of falciparum malaria by bites of *Anopheles quadrimaculatus* and *A. freeborni* and obtained prepatent periods of 15 days and incubation periods of 12 and 18 days in non-immunes. Coatney *et al* (1947) reported that prepatent periods for 31 mosquito-induced infections of falciparum malaria (McLendon strain) ranged from 9 to 13 days with a mean of 11 days; the incubation periods, based on the first temperature of 101° F or higher, ranged from 10 to 15 days with a mean of 12.2 days.

Fairley *et al* (1947) reported prepatent periods ranging from 7 to 12 days (mean 9.5 days) with New Guinea strains of *P. falciparum*. Kitchen (1949) reported a mean prepatent period

of 11 days for 220 naturally induced falciparum infections involving 6 different strains, which included the Costa and the Long strain. The mean incubation period for this same group was 13.1 days. The range for the prepatent periods was 6 to 25 days; for the incubation period, 7 to 27 days. Seventy-five percent of the prepatent periods ranged from 9 to 11 days. Among the 220 infections, only 2, 1, and 1 patients had prepatent periods of 6, 7, or 8 days, respectively.

Eyles and Jeffery (1949) transmitted Santee-Cooper strain falciparum and Panama strain falciparum by bites of *Anopheles albimanus*. With the former the prepatent period was 13 days, and with the latter, 10 to 13 days (median of 11.5 days). Later, Eyles and Young (1951), working with mosquito inoculated Santee-Cooper falciparum, reported prepatent periods from 7 to 13 days. Jeffery *et al* (1952), in their studies on the fixed tissue stages of falciparum malaria, observed prepatent periods ranging from 7 to 13 days after massive exposures to infection, either by mosquito bite or by inoculation of suspensions of sporozoites. The total number of mosquitoes biting ranged up to 8,516, with an 86.8 percent infection rate. The median was 9 days. Jeffery *et al* (1963) in studies with a Thailand strain of resistant falciparum malaria reported prepatent periods of 11 days in 2 control patients who received no medication; and, interestingly enough, prepatent periods as short as 10 days in patients receiving drug suppressively to which this strain was resistant. Of interest, also, was the fact that these infections were in patients all of whom had experienced previous malaria infections. The incubation periods ranged from 15 to 19 days.

Lunn *et al* (1964) and Contacos *et al* (1964), working with a Southern Rhodesian strain of *P. falciparum*, reported prepatent periods of 10 and 11 days and 9 to 19 days, respectively. Powell *et al* (1965) records prepatent periods of 9 days for Thailand and Malayan Camp strains of falciparum. In fact, they observed a single 8-day prepatent period in an individual who had received ineffective antimalarial suppression and/or prophylaxis. Chin *et al* (1967) reported prepatent periods with 3 chloroquine resistant, or multi-resistant, strains of falciparum malaria, which ranged from 9 to

11 days. Contacos and Collins (1968) and Collins *et al* (1968) reported prepatent periods of 11 and 12 days for Malayan IV strain infections.

The studies of Boyd and Kitchen (1937) seemed to indicate that increases in dosages of sporozoites (principally the number of infected mosquitoes biting) 1) did not materially change the proportion of takes, 2) did not appreciably shorten the prepatent period, and 3) did not shorten the incubation period (i.e., elevation of temperature to 100° F or more). In 60 cases with 5 strains, prepatent periods ranged from 6 to 25 days with a median of 11 days. The incubation periods ranged from 7 to 27 days with a median of 12 days. They did observe, however, that the duration of the incubation period tended to vary with the season of the year; being shortest in the 4th quarter (mean of 10.1 days) and longest in the 2nd quarter (mean of 13 days).

As was stated earlier, it has been the intent in this monograph to stress the biologic rather than clinical aspects of primate malaria and, especially, the human malarias. Therefore, for a description of the various clinical "pernicious forms" (cerebral, algid, gastrointestinal, etc.) and blackwater fever (hemoglobinuria), the reader is referred to Boyd's *Malariology* (1949) and James *et al* (1932) for excellent descriptions of these features of falciparum malaria.

In contrast to *P. vivax*, *P. falciparum* infections are considered to be more malignant than the benign vivax. Kitchen (1949) stated that most persons who have had experience with falciparum infections "will attest the capacity of *P. falciparum* both to assume a malignant role and to evoke protean clinical manifestations." Of all the human malarias, this one is potentially the most dangerous.

Boyd and Kitchen (1937) found that falciparum malaria infections exhibit the same general types of febrile reactions that are observed in vivax infections, although pronounced differences did occur. In 60 cases of falciparum malaria, the succession of the different febrile types was not observed as often as in vivax and a larger proportion of the intermittent fevers were tertian. The onset was described, usually, as a remittent fever with a tendency to higher fever peaks. Remittent fever, when present, appears at the onset and may last

for a week or even longer. Its presence suggests a high degree of susceptibility by the patient and/or a greater invasiveness on the part of the parasite. Patients showing a remittent course of fever throughout are more likely to be overwhelmed by the infection unless the fever pattern changes over to an intermittent pattern. Most commonly the infection begins and continues as an intermittent fever which is more often tertian than quotidian in type. The duration of the paroxysms tends to be longer than observed for vivax and the peak of the paroxysms is usually broken into several secondary peaks. In their experience, patients whose clinical onset preceded the appearance of parasites in the peripheral blood may have an abrupt onset, with cerebral symptoms.

Jeffery *et al* (1959) saw that the mean maximum fever varied very little between Panama, McLendon, and Santee-Cooper strain falciparum infections. The mean maximum temperature recorded for McLendon strain was 105.5, for the Panama strain 105.1, and the Santee-Cooper strain 105.0° F. The maximum fever, usually occurred some time between the 5th and 7th day of patent parasitemia. In their experience, the initial fever patterns for each of the 3 strains were quotidian, followed by a tertian, and then a remittent type of fever for the Santee-Cooper and McLendon strains, but a remittent and then tertian pattern for the Panama strain. In many cases of Panama strain falciparum, the fever might more accurately be described as continuous, rather than remittent. These authors stated "it is not surprising that there seems to be some confusion in the literature describing the *P. falciparum* febrile attack. It is almost impossible to describe a 'typical' fever curve for *P. falciparum*." Quotidian periodicity prevailed as the initial pattern in their series of infections. Some of them remained quotidian until the termination of the infection whereas others converted to tertian periodicity. Jeffery *et al* (1959) emphasized that the most prominent characteristic of the fever patterns, in the 3 strains of falciparum studied by them, was extreme variability; in other words, no typical periodicity or pattern could be determined.

Boyd and Kitchen (1937) reported that

there is no apparent limit to the parasite density which may obtain in *P. falciparum* infections; the potentialities of this species for multiplication being so great they regarded daily smears and counts to be essential in following the infections. In our studies, falciparum infections are followed even more closely; namely, blood smears are made, stained, and read every 8 hours to preclude infections getting out of hand.

Boyd and Kitchen (1945) reported that the mean interval from the first day of parasitemia to the day of maximum parasitemia ranged from 4.2 to 11 days for 8 different strains of falciparum malaria; the mean for the exotic strains was 6.7 and the indigenous strains, 8.9 days. The mean maximum parasite counts for these various strains ranged from 11,140 to 369,200 per mm³. The mean for the exotic strains was 83,870 and the indigenous strains 103,950 per mm³.

Coatney *et al* (1947) reported, in 7 patients, that parasite densities went above 100,000 per mm³ of blood. The maximum parasite densities in the primary attacks ranged from 10 to 250,000 per mm³, the latter corresponding to 5 to 10 percent of the red cells. They described 10-fold increases or decreases in the parasite count within a 12 hour period even in the absence of treatment. This calls attention to the fact that in developed infections, low parasite counts are no guarantee of a favorable prognosis. In a Thailand strain (Jeffery *et al*, 1963), maximum parasite counts ranged from 3,394 up to 35,022 per mm³ of blood.

According to Kitchen (1949), a count of 500,000 parasites per mm³ (12.5 percent) probably gives a patient about a 50-50 chance of surviving provided treatment is started immediately and pernicious symptoms do not appear. Field and Niven (1937) showed, by analysis of some 750 cases, that the mortality rate increased greatly as parasite counts increased above 100,000 per mm³ (less than 0.5 percent mortality with counts less than 100,000; 7 to 20 percent mortality with counts ranging from 100,000 to 500,000; and 63 percent mortality with counts over 500,000). Chopra *et al* (1932) reported a patient with more than 50 percent of the erythrocytes parasitized who died within 12 hours of admission. We have observed

a situation where 35 percent of the erythrocytes were parasitized in a Negro patient who became comatose and developed renal failure but survived this malignant experience.

The duration of the primary attack in falciparum malaria, according to Boyd and Kitchen (1937), is shorter than that of vivax malaria, the mean being 10.8 days and the range, 2 to 36 days. However, the data of other workers show that the time of such attacks is extremely variable. Ciuca *et al* (1955) reported parasitemia continuing for up to 27 months, and African strains persist for up to approximately 18 months according to Covell (1960). Some of the American strains have persisted for 503 days. Verdrager (1964) reported falciparum infections of 3 years duration.

Eyles and Young (1951), in summarizing their observations on the duration of *Plasmodium falciparum* infections induced by sporozoites or parasitized blood, reported that following and including one or more clinical attacks, parasites were present continuously in the blood stream for varying periods of time and that the height of parasitemia in these successive waves tended to become lower and lower as time went by. The length of this period of continuous remittent parasitemia had a mean of 121 days with extremes of 32 and 224 days after the beginning of patent parasitemia. They reported further, that the period of continuous parasitemia was followed by a period of intermittent parasitemia which averaged 100 days with a range of 0 to 283 days. The duration of infection appeared to be similar, whether infections were induced by mosquito bites (sporozoites) or by parasitized blood. Three of the infections persisted for more than one year, the longest being 480 days.

Eyles and Young (1951), in studying a group of 13 sporozoite-induced cases, Santee-Cooper strain, observed 9 of them throughout their infections and for 6 months after the last parasites were seen. All 13 infections were observed through the long primary attack with continuous parasitemia. Apparently, 4 had to be treated because of dangerously high parasitemias. They described the general pattern of the infections as characterized by a clinical attack which varied from practically

asymptomatic to one of severe dimensions. The clinical period was followed by an asymptomatic period during which patients carried parasites in their blood continuously. This was then followed by a period of varying duration during which parasites were only intermittently observed in peripheral smears. The mean length of the initial clinical episode was 9 days and the mean number of clinical episodes was 1.4. The mean total hours of fever, over 101.0° F, orally, was 90.4. The median maximum parasitemia was 65,000 per mm³ of blood. The fact that only one clinical episode was observed in most cases and the fact that two-thirds of their original 13 cases were able to terminate their clinical symptoms spontaneously indicated to them that they were working with a strain of low virulence.

Jeffery and Eyles (1954) carried out studies on the Panama strain of *P. falciparum*. Of the 24 sporozoite-induced infections, 12 had attacks which had to be partially suppressed. The mean length of the initial clinical episode was 13.1 days; the mean number of clinical episodes was 3.3. The mean total hours of fever, over 101.0° F, was 124.8. The median maximum parasite count was 49,121 per mm³; the mean maximum parasite count was 73,741 per mm³. The general pattern of this strain of malaria was very similar to that of the Santee-Cooper strain (Eyles and Young, 1951). Probably the most significant difference was the greater severity of the primary clinical period observed with the Panama strain; its clinical attack was described as being quite severe. The mean length of the period of continuous remittent parasitemia for the Panama strain was 115.7 days with a range of 36 to 220 days. The terminal period of intermittent parasitemia had a mean duration of 168.3 days. The mean total duration of infection was 279 days with a range of 114 to 503 days.

Boyd and Kitchen (1937) observed renewal of clinical activity after spontaneous cessation of the primary attack or subcurative therapy of the primary attack in, roughly, 58 percent of their cases. Renewal of clinical activity was observed as many as 4 times in some patients. Most of the recurrences were observed within a period of 8 weeks following the primary attack. Kitchen (1949) reported recrudescences within 2 months

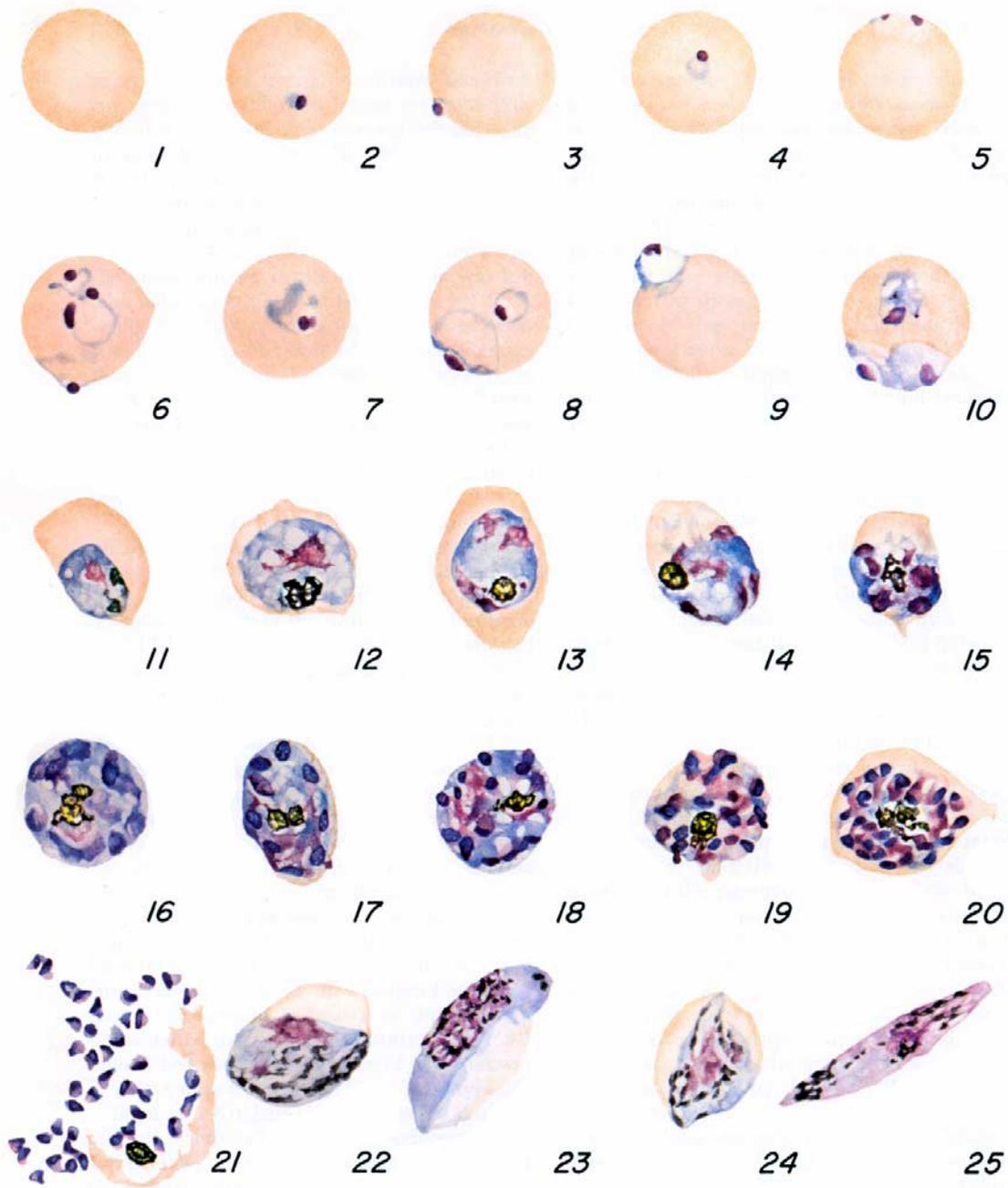
(8 weeks) of the termination of clinical activity but did not observe any clinical reactivation after 24 weeks; whereas James *et al* (1932) reported secondary clinical attacks in 3.3 percent of their infections after 24 weeks. In our studies with drug resistant strains of falciparum malaria, we have found that recurrences (recrudescences) could occur up to 80 days after subcurative or ineffective antimalarial therapy. For this reason, we require a 90-day period of follow-up before recording a cure.

Since 1960, a large number of strains of falciparum malaria have been found resistant to antimalarial drugs. They are resistant not only to chloroquine and other quinolines, but also to one, or more, or all, of the synthetic antimalarials, including chlorguanide, pyrimethamine, and mepacrine. In addition, some strains from Southeast Asia have shown varying degrees of resistance to quinine.

The susceptibility of the chimpanzee to falciparum malaria has been studied by several workers. Mesnil and Roubaud (1920) failed to infect these animals when exposure was by the bites of infected mosquitoes. Lefrou and Martignoles (1954) demonstrated the persistence of *P. falciparum* parasites for as long as 3 weeks in 3 of 4 chimpanzees inoculated with blood containing the parasite. However, Bray (1958, 1960) studied the susceptibility of the chimpanzee to falciparum malaria in detail and showed that the sporozoite was able to develop in the parenchymal cells of the liver. Although patent parasitemia obtained in the intact chimpanzee, it did not persist for more than one or, at the most, 2 cycles. However, if a splenectomized chimpanzee was exposed to infection by the bites of infected mosquitoes, the blood stages appeared, developed, and multiplied rapidly; the gametocytes failed to reach maturity (Bray, loc. cit.; Rodhain and Jadin, 1964).

In the *Aotus trivirgatus* monkey, *P. falciparum* often produces very high parasitemias. Geiman and Meagher (1967) reported a peak parasitemia, in a splenectomized monkey, on the first passage from man, of 180,000 per mm³. Subsequent passage into splenectomized *A. trivirgatus* monkeys produced even higher peak parasitemias, some reaching

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J. H. Nicholson

PLASMODIUM FALCIPARUM IN AOTUS TRIVIRGATUS

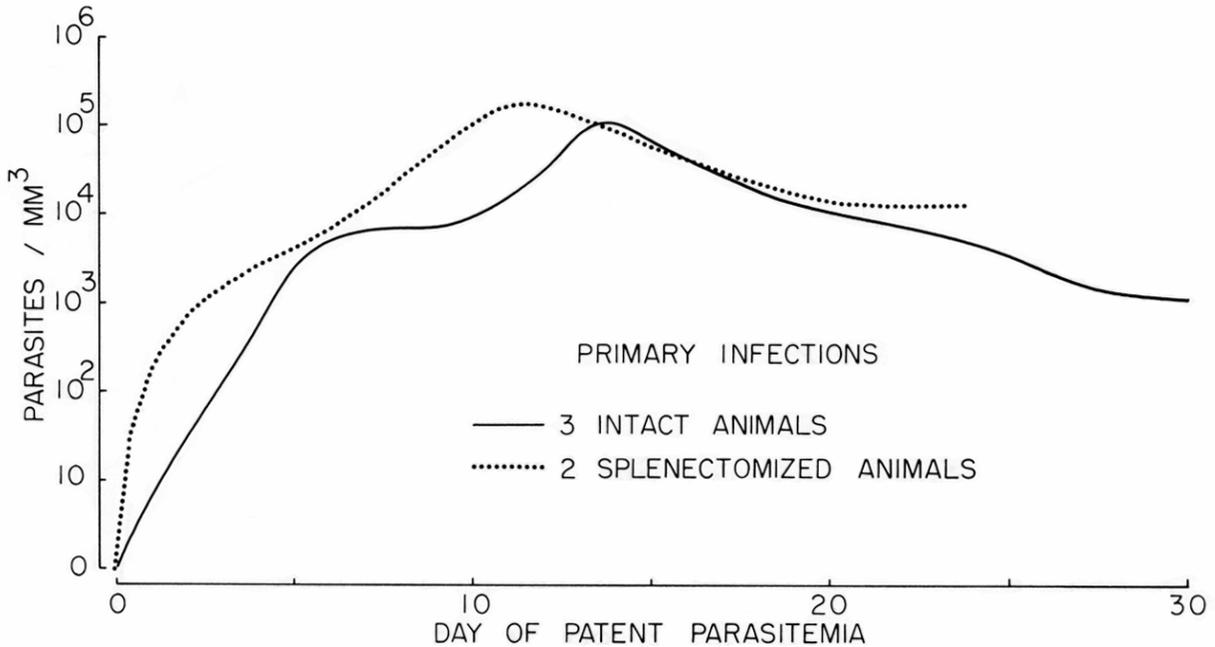


FIGURE 58.—Median parasitemia curves of *Plasmodium falciparum* infections in *Aotus trivirgatus* monkeys infected by the intravenous inoculation of parasitized blood from man.

levels as high as 87 percent of the red cells being infected (Geiman *et al*, 1969).

In our studies, *P. falciparum* has been passed from man to the *A. trivirgatus* monkey on 5 occasions, in intact animals 3 times, and in splenectomized animals, twice (see Plate XLIV). We have elected to term sub-passages in monkeys as secondary infections. The primary infections had similar courses of parasitemia in both the splenectomized and the intact monkeys (Fig. 58). On subsequent passage (Fig. 59), peak parasitemias of approximately 300,000 per mm^3 obtained in the splenectomized monkeys by day 8 and in intact animals, by day 14. The number of parasites in the inoculum greatly affected subsequent parasitemia in intact *A. trivirgatus* monkeys (Fig. 60). During the first, or primary,

passage from man to intact monkeys, peak parasitemias of approximately 70,000 per mm^3 were obtained after 14 days of patent parasitemia. Secondary infections, initiated by a small number of parasites given to intact monkeys, resulted in peak parasitemias of approximately 300,000 per mm^3 on patent parasitemia day 14 and subsequently declined. However, if a large number of parasites were given, the parasitemia reached a peak of approximately 1,000,000 per mm^3 by day 10 after which the animal, in most cases, died; some were saved from death by administering antimalarial drugs.

The gametocytes in *A. trivirgatus* monkeys were readily infectious to mosquitoes (Contacos

PLATE XLIV.—*Plasmodium falciparum* in the owl monkey, *Aotus trivirgatus*.

Fig. 1. Normal red cell.

Figs. 2-5. Young trophozoites.

Figs. 6-10. Growing trophozoites.

Figs. 11, 12. Mature trophozoites.

Figs. 13-18. Developing schizonts.

Figs. 19-21. Nearly mature and mature schizonts.

Figs. 22, 23. Mature macrogametocytes.

Figs. 24, 25. Mature microgametocytes.

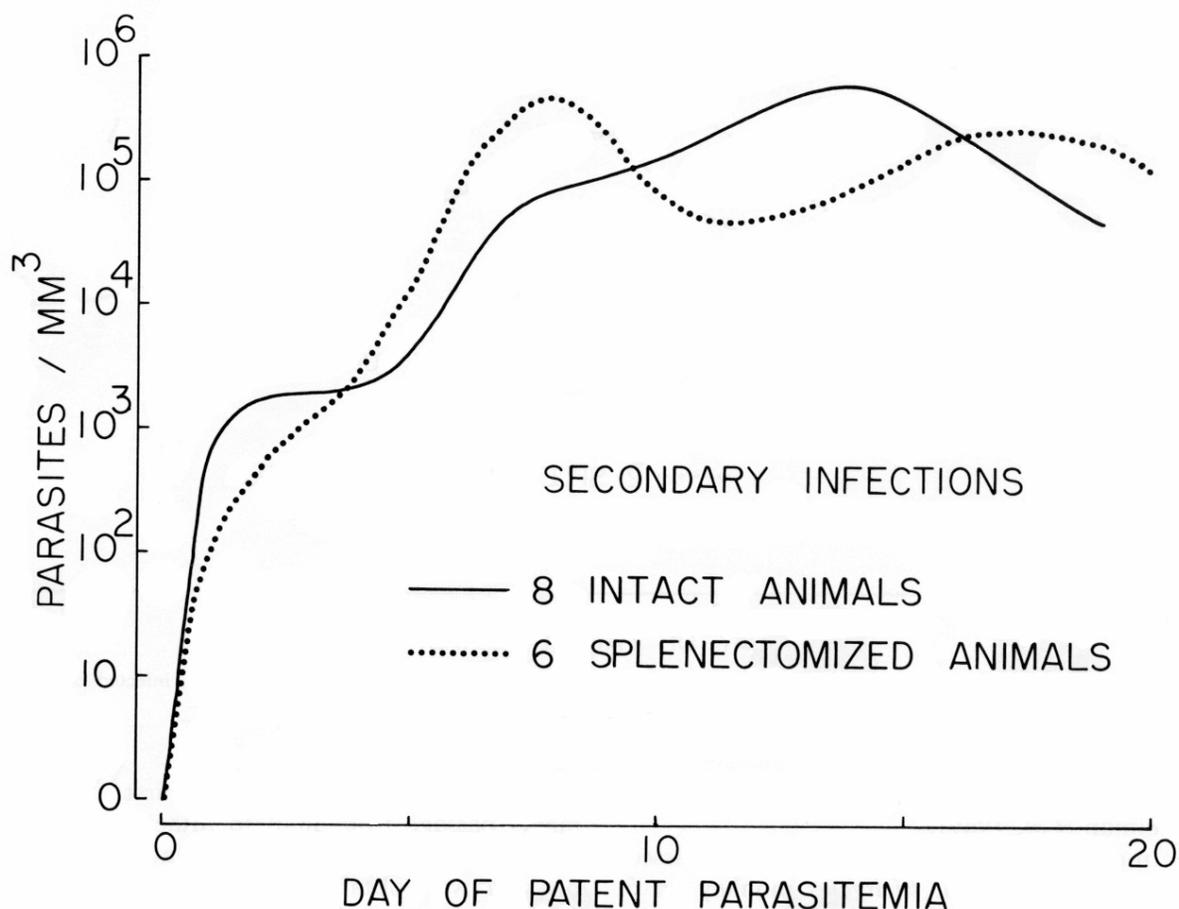


FIGURE 59.—Median parasitemia curves of *Plasmodium falciparum* infections in *Aotus trivirgatus* monkeys infected by the intravenous inoculation of parasitized blood from other *A. trivirgatus* monkeys.

and Collins, 1968; Collins *et al*, 1968). This is illustrated in Figure 61 where *Anopheles freeborni* mosquitoes were allowed to feed on a monkey infected with a Malaysian strain of *P. falciparum*. This animal (AO-23) had been infected by blood-passage from man. After a low, transient course of parasitemia, the animal was able to eliminate the infection. Following splenectomy, the monkey was re-inoculated with the same strain of parasite. Eight days after inoculation, the first mosquito infections were obtained and these continued for the next 40 days. On 16 of the days, the infection rate was 100 percent and, in many instances, the number of oocysts per gut exceeded 500.

Young and Baerg (1969) observed infections of *P. falciparum* in the white faced

monkey, *Cebus capucinus*. The animals were infected by the inoculation of parasitized blood from infected *A. trivirgatus* and *C. capucinus* monkeys. The prepatent periods ranged up to 30 days; the periods of patent parasitemia up to 72 days. Infections reached high parasitemia levels in 3 of 8 monkeys; the highest was 662,700 per mm^3 . In all cases but one, the infection was self-limiting.

In the squirrel monkey (*Saimiri sciureus*), *P. falciparum* infections are remarkably transient. Young and Rossan (1969) reported an infection in an intact monkey which persisted at a detectable level for 21 days with a maximum parasitemia of 2,210 per mm^3 . After 49 days, parasites were again demonstrable for 3 consecutive days, but were not seen again. On

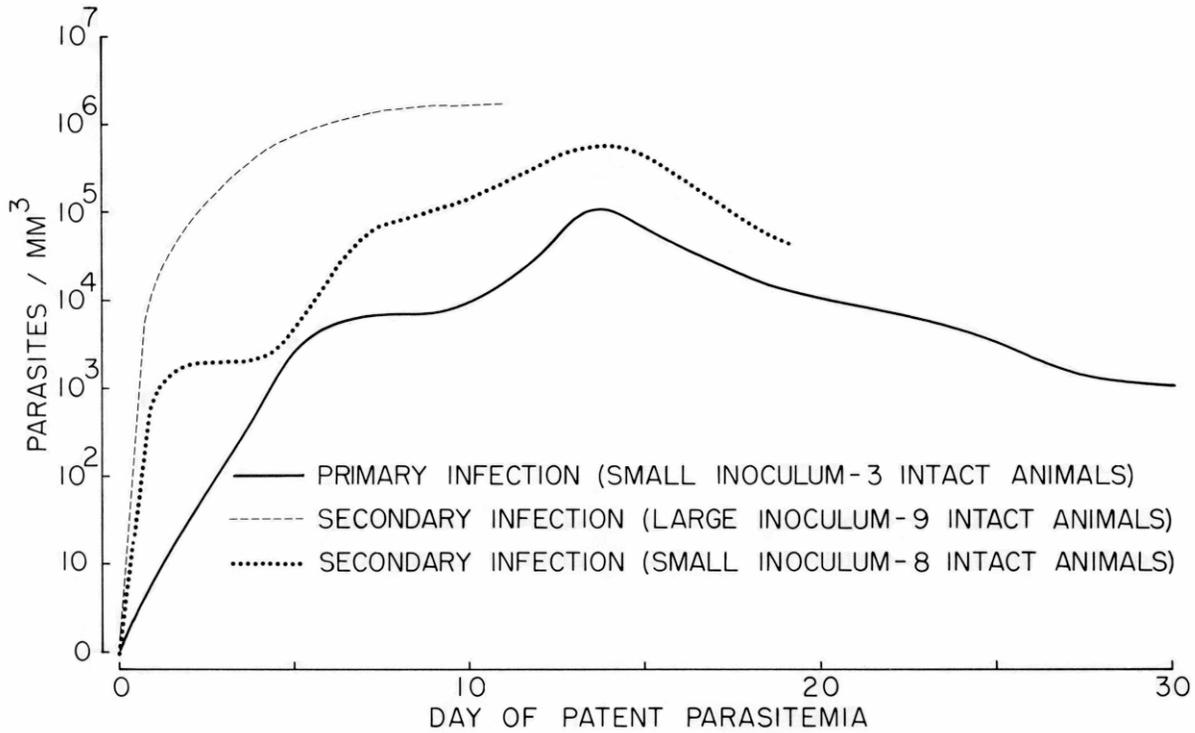


FIGURE 60.—Median parasitemia curves of *Plasmodium falciparum* infections in *Aotus trivirgatus* monkeys infected by the inoculation of parasitized blood.

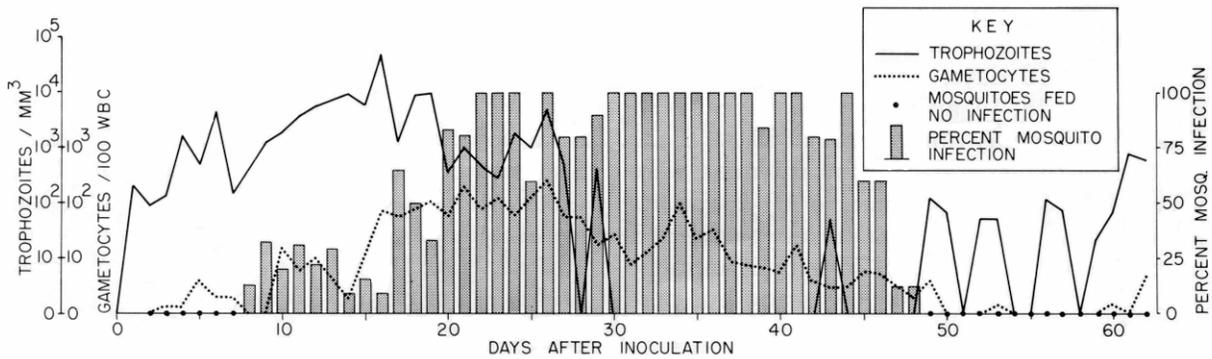


FIGURE 61.—Parasitemia and infectivity of *Anopheles freeborni* mosquitoes to a strain of *Plasmodium falciparum* from Malaysia (Malayan-IV in an *Aotus trivirgatus* monkey).

one occasion we gave a heavy inoculum to a splenectomized *S. sciureus* monkey; 3 days later, the parasite count was approximately 120,000 per mm³. This high level was maintained for approximately 2 weeks; 20 days after infection, the parasite count had dropped to 15,400 per mm³. Thereafter, the parasitemia declined rapidly; parasites were last seen 29

days after inoculation. Upon reinoculation, the animal experienced a low, transient parasitemia. When mosquitoes were allowed to feed, they became infected but the infection was low.

Porter and Young (1967) recorded infections of *P. falciparum* in the marmoset, *Saguinus Geoffroyi*. In 4 intact animals, inoculated with parasitized blood, patent

infections were seen in from 1 to 2 days; the periods of patent parasitemia ranged from 4 to 15 days. The maximum parasite count was 22,660 per mm³.

Gibbons, *Hylobates lar*, have also been shown susceptible to infections with *P. falciparum* (Ward *et al*, 1965; Ward and Cadigan, 1966; Gould *et al*, 1966; Cadigan *et al*, 1969). In studies on a large number of animals, these workers found that once the infection became patent, the parasite levels rose rapidly to one percent of red cells infected or higher; after about 2 weeks, the parasite counts began to fall. Subsequent rises occurred, but the parasite counts tended to diminish with succeeding waves. The median duration of detectable parasitemia was approximately 31 weeks. In individual animals, this varied from 6 weeks to as long as 72 weeks. The infections did not produce overt disease. Experimentally, splenectomized gibbons can be infected by the inoculation of sporozoites (Gould *et al*, 1966). Attempts to infect mosquitoes were unsuccessful.

Cadigan *et al* (1966) reported the successful infection of splenectomized *M. mulatta siamica*, *M. nemestrina*, and *M. irus* (= *fascicularis*) monkeys with *P. falciparum* which had been adapted to the gibbon, *H. lar*. After periods of 14, 6, and 10 days, detectable parasitemias persisted for 18, 19, and 16 weeks, respectively. Peak parasitemias ranged from 850 to 2,420 per mm³. In general, the parasitemias were very low. Passages from man to *M. fascicularis* monkeys by the inoculation of parasitized blood produced infections in 4 of 5 animals. The parasitemias were of low grade but persisted for as long as 19 weeks.

Host Specificity

Plasmodium falciparum naturally infects man only. Experimentally, however, infections have been obtained in a number of primates. Early attempts to infect chimpanzees with *P. falciparum* resulted in failure (Mesnil and Roubaud, 1920; Blacklock and Adler, 1922; Rodhain, 1939). Bray (1958), however, obtained infections in chimpanzees (*Pan troglodytes versus*) by the intravenous inoculation of infected salivary glands. In intact animals, the

parasitemia was transient. In splenectomized animals, however, the parasitemia not only persisted but exhibited all forms of the schizogonic cycle in the peripheral blood. Rodhain and Jadin (1964) also reported the infection of splenectomized chimpanzees but the gametocytes were unable to develop to maturity. Ward *et al* (1965), Ward and Cadigan (1966), Gould *et al* (1966), and Cadigan *et al* (1969) found splenectomized gibbons (*Hylobates lar*) susceptible to *falciparum* infection either by sporozoites or by the inoculation of parasitized blood.

Probably the most exciting results relating to *P. falciparum* in non-human primates, in terms of practical laboratory studies, are those involving South American monkeys. Taliaferro and Taliaferro (1934) and Taliaferro and Cannon (1934) were able to infect brown howler monkeys, *Alouatta palliata* (= *fusca*) by the inoculation of parasitized blood from man. However, we had to wait for over 3 decades before Geiman and Meagher (1967), Contacos and Collins (1968), Collins *et al* (1968), Geiman *et al* (1969), and Voller *et al* (1969) showed that splenectomized and intact owl monkeys, *A. trivirgatus*, were highly susceptible to infection with *P. falciparum*. In 1969, Sodeman *et al* demonstrated that EE bodies would develop only partially in the owl monkey and that blood infections did not result from sporozoite inoculation. Infections have also been obtained in the white faced monkey, *Cebus capucinus*, (Young and Baerg, 1969), the squirrel monkey, *Saimiri sciureus*, (Young and Rossan, 1969), and the marmoset, *Saguinus geoffroyi*, (Porter and Young, 1967),

Cadigan *et al* (1966) reported low-order infections in splenectomized *M. mulatta siamica*, *M. nemestrina*, and *M. irus* (= *fascicularis*).

A great number of anopheline mosquitoes have been shown to be natural or experimental hosts for *P. falciparum*. Garnham (1966) lists 66 species as hosts for this parasite, and no doubt, many more are susceptible to infection. We have made only limited studies in this area. Among those we have examined (Table 35), *A. freeborni* was the most susceptible with either the McLendon or Malayan IV strains. These results and those of our previous studies (Collins, 1962;

Collins *et al*, 1963, 1964) have confirmed the opinion of many investigators that the infectivity of isolates of *P. falciparum* to different anophelines is dependent to some extent on the geographical origin of either the parasite or the mosquito. This is well illustrated in Table 35 in which it is shown that with the McLendon strain from southern United States, the gut infection index ratio between *A. freeborni* and *A. quadrimaculatus* was 100:38, whereas with the Malayan IV strain from southeast Asia, the ratio was 100:4.3. The results of comparative infectivity studies are so variable between different isolates of *P. falciparum* that it is often necessary to feed a number of species on the host of a "new" isolate in order to determine which of the species available will serve as suitable experimental vectors. For example, with the Panama strain of *P. falciparum*, *A. albimanus* mosquitoes are very good hosts (Jeffery *et al*, 1950), but with the Thailand strain, it is almost refractory (Collins *et al*, 1963).

Immunity and Antigenic Relationships

There appears to be no racial or innate immunity among Negroes against falciparum malaria as has been observed for vivax malaria. The results obtained by Kitchen (1949), when

analyzed according to race, suggested that race did not play a significant role in regulating and/or modifying the prepatent or incubation periods. However, the Negro does have a tendency to be able to clinically tolerate falciparum malaria infections better than the Caucasian.

The presence of genetic traits which inhibit parasite multiplication or survival, such as sickle cell hemoglobin and enzyme deficiencies, have been reported but are not universally accepted. It has been suggested, that possession of the sickle cell trait or sickle cell character has a "tolerating effect" on the course of falciparum malaria in individuals with these hemoglobin types; in other words, individuals with sickle cell type hemoglobin are rather poor hosts for *Plasmodium falciparum*.

Allison (1954) and Mackey and Vivarelli (1954) suggested that sickling hemoglobin, present in erythrocytes of carriers of sickle cell trait, was not as favorable for the development of malaria parasites as normal hemoglobin. Allison (1954a, 1961, 1963) obtained good correlation between the incidence of sickle cell trait and the degree of malarial infection. Regions in East Africa, where malaria is hyperendemic, had a higher incidence of sickle cell trait than did regions where malaria was

TABLE 35.—Comparative infectivity of two strains of *Plasmodium falciparum* to different species of *Anopheles*.

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
McLENDON STRAIN						
F-1						100
F-1 : Q-1	8	100	131	86.0	78.6	38.0
F-1 : Alb	12	300	175	42.7	29.1	32.5
F-1 : Mac	3	45	57	100.0	79.2	23.8
MALAYAN IV STRAIN						
F-1						100
F-1 : St-1	6	81	127	35.8	19.7	28.8
F-1 : Mac	6	56	111	60.7	9.0	13.4
F-1 : Q-1	10	147	223	37.4	5.4	4.3
F-1 : Bal	8	147	136	37.4	11.8	2.0

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

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

epidemic or absent. Allison suggests that the sickle cell trait may confer a certain degree of resistance against malaria and, for that reason, the trait survives more successfully where the malaria infection rates are more severe.

It is thought, in some quarters, that the parasite cannot effectively utilize the abnormal hemoglobin in the sickled cell. Mackay and Vivarelli (1954) reported that sickle cells were seldom parasitized in a blood film from an individual with sickle-cell trait even when the sickle cells predominated. Miller *et al* (1956), on the other hand, reported results contrary to those of Mackay and Vivarelli; namely, that falciparum malaria parasites can and do, enter and develop readily in cells which undergo sickling. In 2 of their 3 cases, parasites were found as frequently in sickled cells as in nonsickled cells.

Miller *et al* (1956) suggested that it was not necessarily the sickle cell hemoglobin alone which was detrimental to the development of the falciparum parasites, but other factors were functioning, too. When the parasitized red cells, containing sickle cell hemoglobin, adhere to the walls of blood vessels, as falciparum infected red cells have a tendency to do, the oxygen supply to these parasitized cells would be very much decreased; and, under such circumstances, the loss of oxygen would bring about a relative anoxia which could conceivably induce sickling of the parasitized erythrocyte, thereby interfering with the multiplication of the parasite.

Another genetic trait which acts adversely is the deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD). High frequencies of this deficiency are found in malarious areas, and especially, in those endemic for falciparum malaria. It has been suggested that such a deficiency may limit parasite multiplication. Allison and Clyde (1961) found a significant lowering of the parasite rate in males deficient in G6PD and the proportion of falciparum infections with parasite counts above 1,000 per mm³ was significantly lower in G6PD deficient children than in normal children.

Acquired immunity to falciparum malaria, as with the other human malarias, is strain specific. Garnham (1966) stated that *P.*

falciparum parasites induce "a highly effective degree of premunition in the indigenous host in the sense that the children look thoroughly healthy, although 80 percent or more have a heavy load of parasites in the blood, while the adults appear strong, well grown, and energetic, with a relatively low parasitemia." However, if residents of the endemic areas move to other endemic areas, and are exposed to new strains, they develop very heavy infections.

Most studies have indicated that immunity to malaria is a residual immunity acquired through infection and that this immunity is not life-long. Rather, it is usually of short duration unless the individual is infected repeatedly. Boyd *et al* (1936) concluded that homologous but not heterologous immunity is acquired through infections of *P. falciparum*. This is contrary to what they observed with vivax malaria. In falciparum malaria, they found that reinoculation with a different strain resulted in an infection oftentimes as severe as the first. The homologous immunity lasted approximately 4 months. The latter is generally characterized as a clinical immunity; namely, no clinical attack with or without low patent parasitemia--if you will--an asymptomatic attack. The degree of immunity, acquired through infection, is probably dependent on the parasite densities and the duration of the parasitemia. Ciuca *et al* (1934) observed that repeated inoculations with falciparum malaria progressively increased the degree and/or the duration of immunity.

Boyd *et al* (1939) recorded absence of cross-immunity between vivax and falciparum malarias whether the reinoculations were effected during the incubation period, the acute primary attack, or shortly after termination of that attack.

However, Boyd and Kitchen (1945) reexamined and modified their opinions concerning heterologous immunity between falciparum malarias. They stressed the fact that the primary attacks of falciparum malaria were more severe in Caucasian than in Negro patients. In Caucasians, the primary infections usually consisted of 2 or 3 successive waves of patent parasitemia whereas in Negro patients only one wave of patent parasitemia was commonly observed. Generally, the heterologous immunity was characterized by a shortened period of

clinical activity. In addition, reinoculation of Caucasian patients with heterologous strains resulted in infections similar to the primary infections in Negro patients; namely, one wave of patent parasitemia with parasite densities lower in the Negro patients. Their final conclusion was that immunity acquired from a falciparum malaria infection has "appreciable heterologous value."

That humoral immunity exists in falciparum malaria was shown by Cohen *et al* (1961) and Cohen and McGregor (1963). They demonstrated that malarial immunity can be transferred passively in the 7S fraction of gamma globulin of hyperimmune serum from adults in hyperendemic areas of the Gambia. When they administered such gamma globulin preparations in large doses to acutely ill Gambian children with heavy infections, a consistent pattern of response was observed; namely, rapid clinical recovery and a highly significant reduction in parasitemia. This was confirmed by Edozien *et al* (1962) in Nigeria. McGregor (1964) was of the opinion that immune 7S gamma globulin acted against the late asexual forms (schizonts) or the liberated (extracellular) merozoites.

That humoral malarial immunity could be passively transferred from mother to offspring had been postulated for years. This was confirmed by Edozien *et al* (1962) when they demonstrated that antimalarial antibodies (gamma globulins) can cross the placental

barrier when they treated falciparum infections with gamma globulin prepared from cord blood.

If one turns to serology for insight into relationships between various primate malarias, some interesting facts emerge. In this regard, Kuvin and Voller (1963) studied the differences in response of sera from 26 individuals from West Africa, previous malarial histories unknown, against *P. falciparum* and B strain *P. cynomolgi* antigens. The mean titers of the former were 1:24 versus 1:18 for *P. cynomolgi* indicating a high level of cross-reactivity. Diggs and Sadun (1965), using sera from known infections of *P. falciparum* and *P. vivax*, quantitated the levels of cross-reactivity. Their *P. falciparum* antisera gave a homologous response, as measured by the geometrical mean of the reciprocal titers, of 1:28.2 and a heterologous response of 1:6.3. Using the *P. vivax* antisera, the homologous geometrical mean of the reciprocal titers was 1:17.2 and the heterologous mean titer was 1:9.3. Collins *et al* (1966) were able to show that antiserum to *P. falciparum* would react with a number of simian malaria antigens but that the highest heterologous response was to *P. fieldi*. Meuwissen (1968) demonstrated that although the antisera to *P. falciparum* would react at a high level to both the B strain *P. cynomolgi* and *P. fieldi* antigens, the highest heterologous response was to the latter.

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(NS) = Not seen.