

Plasmodium malariae (Grassi and Feletti, 1890)

SYNONYMS:

Haemamoeba malariae Feletti and Grassi, 1889; *Plasmodium malariae* M. and C. var. *quartanae* Celli and Sanfelice, 1891; *Plasmodium malariae quartanae* Kruse, 1892; *Haemamoeba laverani* var. *quartanae* Labbe, 1894; *Haemosporidium tertiana* Lewkowicz, 1897; ? *Plasmodium rodhaini* Brumpt, 1939.

There is little doubt that Laveran saw this parasite in fresh blood of patients in Algeria in 1880 because his illustrations show schizonts with 8 merozoites arranged in the typical rosette and a central body of pigment. In his opinion, the human malaria parasites belonged to one species and, possibly for that reason, he did not propose a name. He recognized the phenomenon of periodicity but rejected the idea that it might be a clue toward the taxonomy of the malarias.

Golgi in a short note to the Royal Academy of Medicine in Turin (1885) first recognized the periodic succession of fever attacks and in 1889 he clearly showed how to separate tertian and quartan fevers by linking the development of a particular brood of parasites to the fever episode. He was definite in pointing out that if only young forms are present in the circulating blood there will be 1 or 2 days, depending on the species, free of fever. Golgi was also able to demonstrate quotidian fevers in both tertian and quartan infections and postulated, correctly, that the phenomenon was due to double and triple broods of parasites. This important contribution to the biology of these parasites was more or less ignored at the time and then virtually forgotten until well after the turn of the century.

In attempting to arrive at the correct name and credit for the quartan parasite of man, one runs into considerable difficulties. Coatney and Young (1941) in reviewing the problem arrived at the conclusion that the name *Plasmodium*

malariae should be declared valid as the *de facto* name and credited to Grassi and Feletti, 1890. Part of this was accomplished, Opinion 283, under the Plenary Powers of the Commission on Zoological Nomenclature (Hemming, 1954), but credit for the name was given to Grassi and Feletti, 1889. It now appears that the 1889 pamphlet, which is said to have given the authorship in reverse, is no longer extant and therefore the order of authorship cannot be verified. In 1890, Grassi and Feletti gave *malariae* as the specific name for the quartan parasite in the genus *Haemamoeba*, and on the basis of priority, that date is valid. Following this, Grassi and Feletti (1892) described and illustrated the quartan parasite, as seen in the peripheral blood, and separated it from the other blood-inhabiting forms in man and birds under the name *H. malariae* Grassi and Feletti. Garnham (1966) holds that "in view of the authority of this paper, and the disappearance of all traces of the original 1889 pamphlet", the name should be credited to Grassi and Feletti 1892. The tenet that authority is a valid basis for assigning credit is unacceptable, and in view of the loss of the 1889 paper and the fact that the Regles had to be suspended in order to validate the *de facto* name, it appears to us, therefore, that the correct name for the human quartan parasite is *Plasmodium malariae* (Grassi and Feletti, 1890).

Plasmodium malariae is a cosmopolitan parasite which develops where the summer isotherm does not fall below 15° C (59° F). Its distribution is variable and spotty. Why this is true no one has been able to explain although numerous theories have been advanced; it is still, one of the unsolved problems in the biology of human quartan malaria.

One theory to account for its unique distribution was that because the parasite

requires an extended sporogonic cycle, its greatest prevalence would be in areas where the vector was able to survive for the longest time. This might obtain in some areas, but it sometimes reaches its highest incidence in parts of the tropics where the vector has only a short life.

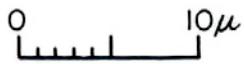
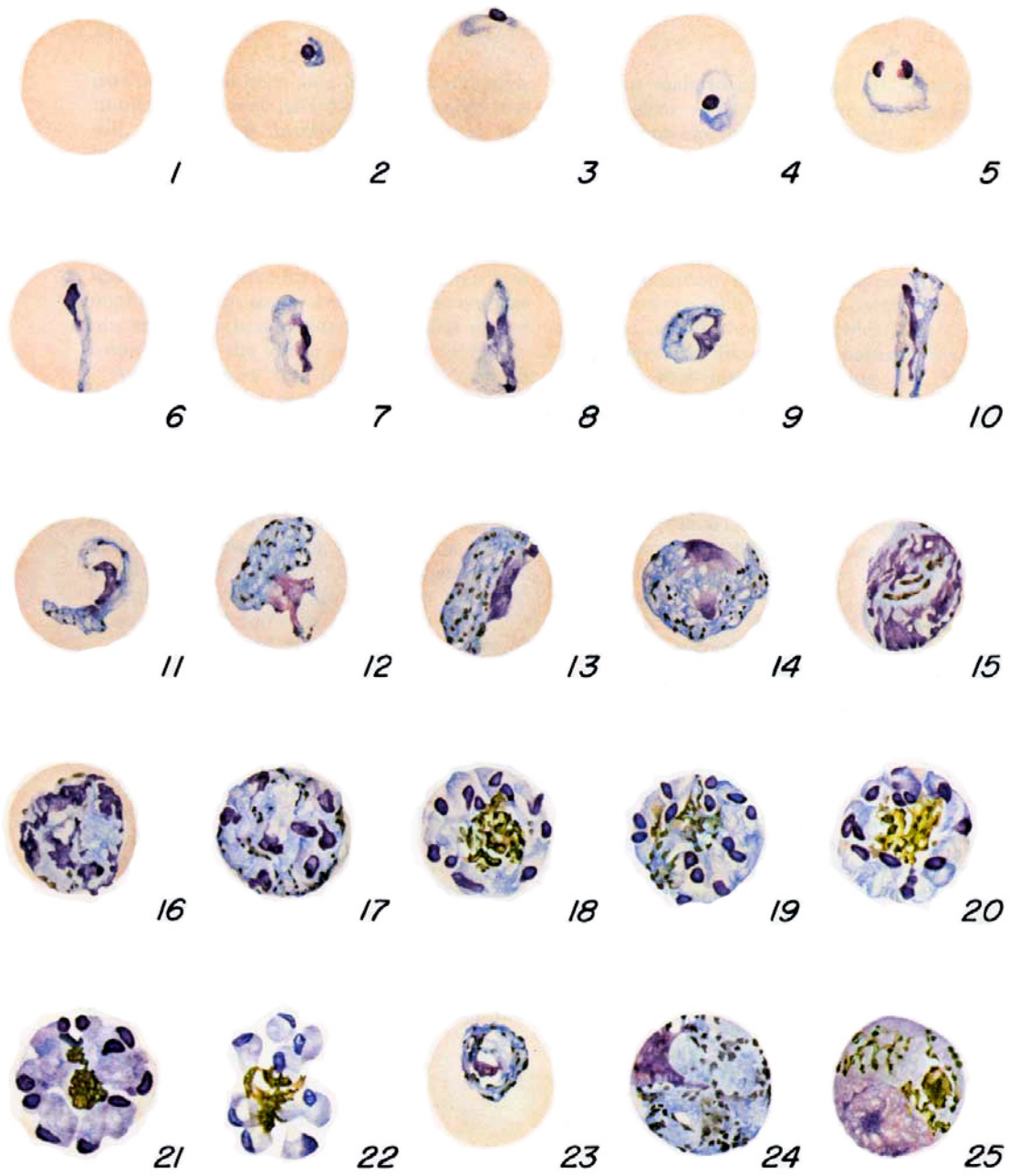
Another theory was that quartan malaria demands a special vector. It is true, that experimental infections in mosquitoes are difficult to produce and when obtained, the oocysts fail to develop at a uniform rate which probably accounts for the varied results obtained by such workers as Mayne (1932), Mer (1933), and others. However, the special vector theory hardly seems to cover the problem either because many species transmit the parasite: *A. atroparvus*, *A. sacharovi*, *A. stephensi et al.*

The third theory, rested on the presence of an animal reservoir in certain tropical regions. This might be applicable if one is concerned with certain areas in tropical Africa where chimpanzees harbor *P. rodhaini*. In man, that parasite takes on the attributes of *P. malariae* and, in some quarters, is considered to be *P. malariae*. The *inui* parasites of India and the Malaysian area are morphologically distinct from human quartan and so far only one strain has been established in man. The rest of the Old World is without any zoonotic connection to explain the persistence of quartan malaria. In the New World, the situation is altogether different than it is in Africa or the Far East, because it appears relatively clear that the quartan malaria of monkeys (*P. brasilianum*) came from man, hence an anthroponosis, rather than the other way around.

It may well be that one or more of these factors, along with changes in the environment which might facilitate transmission, play a part in the unique distribution of quartan malaria throughout the world. But whatever the explanation may be, it appears that *P. malariae* is probably the oldest parasite in terms of time, and although Knowles *et al* (1930) considered it a disappearing parasite, it might well be that instead of dying out, it has 'learned' during its long association with man how to cope with adversity and, when conditions permit, to enjoy prosperity.

This is illustrated in the work of Field and Shute (1956) where they site that in West Malaysia "quartan infections are not commonly found in hospital patients--less than five per cent of the admissions . . .--but there are localized areas where *P. malariae* is the dominant species." In areas of high transmission of all species, *P. falciparum* is dominant for a few months, whereupon, it is succeeded by *P. vivax*, which holds center stage for a time only to be replaced by *P. malariae* which has 'learned' to wait in the wings. Field and Shute call it a "residual infestation" which pretty well sums up the life-cycle of *P. malariae*. It is a highly successful parasite in that it can live in a host longer than any other malaria and without renewed activity from fixed tissue parasites, it causes the host relatively little inconvenience because of its symbiotic nature and, it is always ready, during periods of recrudescence, to gain access to a cooperative vector and, hence, perpetuate the species.

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H. H. Nicholson

PLASMODIUM MALARIAE

Cycle in the Blood

PLATE XXXIII

The first stage to appear in the peripheral blood is the familiar ring-stage, not shown as a true ring on the plate, a circle of cytoplasm, and a spherical bit of chromatin enclosing a vacuole. It very shortly occupies one-fourth to one-third of the parasitized cell and sometimes exhibits an accessory chromatin dot (Figs. 4, 5). Although this parasite generally displays denser cytoplasm and chromatin than *P. vivax*, it is difficult to separate them at this stage because they are about the same size. The parasite grows more slowly than any of the other human malarial parasites. The vacuole disappears after a few hours. In the living condition, the movement is sluggish. Pigment granules appear early in its growth and sometimes a granule may appear in the late ring-stage. The pigment increases rapidly; half grown parasites may exhibit 30 to 50 jet-black granules (Figs. 12, 13) in contrast to the rod-like pigment found in *P. vivax*.

As the parasite grows, it assumes various shapes. Some appear stretched out like a ribbon across the host cell and are known as band forms (Figs. 6, 10, 11). Band forms are found in other species, but are more frequent in *P. malariae* and hence are considered diagnostic. These forms may be seen at any time until the parasite virtually fills the host cell which is not enlarged or blanched. At about the 54th hour, segmentation begins, and by the 65th hour, the host cell is completely filled, or nearly so, and the parasite contains 5 to 6 chromatin masses; the pigment is scattered (Figs. 14-16). During further growth, the definitive number of nuclei are formed and with the final number, the cytoplasm divides to give each nucleus a small amount of cytoplasm. During this stage, the pigment may appear segregated and then

clumped in a loose mass in the center of the cell, surrounded by more or less symmetrically arranged merozoites, to give the "rosette" effect so characteristic of the species. The number of merozoites may be from 6 to 12, sometimes 14; the average is 8 (Figs. 17-22).

The young gametocytes are very similar to the asexual forms which makes it virtually impossible to distinguish them with certainty until about the 54th hour when the asexual forms begin segmentation (Fig. 23). The mature macrogametocyte exhibits a heavy deeply-staining blue cytoplasm with a small, eccentric, well-defined deep red-staining nucleus. The pigment is scattered. The parasite completely fills the host cell (Fig. 24). The cytoplasm of the adult microgametocyte takes a light bluish-pink stain. The pigment is limited to this area of the parasite. The nucleus is diffuse, takes a pinkish-blue stain, and may occupy about half the cell. The parasite fills the entire host cell (Fig. 25). Ordinarily, microgametocytes outnumber the macrogametocytes but this may vary with different strains.

The asexual cycle requires 72 hours.

Sporogonic Cycle

PLATE XXXIV

Observations have been made by a number of workers concerning the sporogonic cycle of *Plasmodium malariae*, but Shute and Maryon (1952) carried out the first definitive studies. These investigators observed its development in *Anopheles atroparvus* mosquitoes incubated at a temperature of 25°C. They found that the pigment, which seldom consisted of more than 30 granules, was very dark brown and variable in size. During the first 7 to 8 days, the granules were distributed over the oocyst, but, from the

PLATE XXXIII.—*Plasmodium malariae*.

Fig. 1. Normal red cell.

Figs. 2-5. Young trophozoites.

Figs. 6-11. Growing trophozoites.

Figs. 12, 13. Nearly mature and mature trophozoites.

Figs. 14-20. Developing schizonts.

Figs. 21, 22. Mature schizonts.

Fig. 23. Developing gametocyte.

Fig. 24. Mature macrogametocyte.

Fig. 25. Mature microgametocyte.

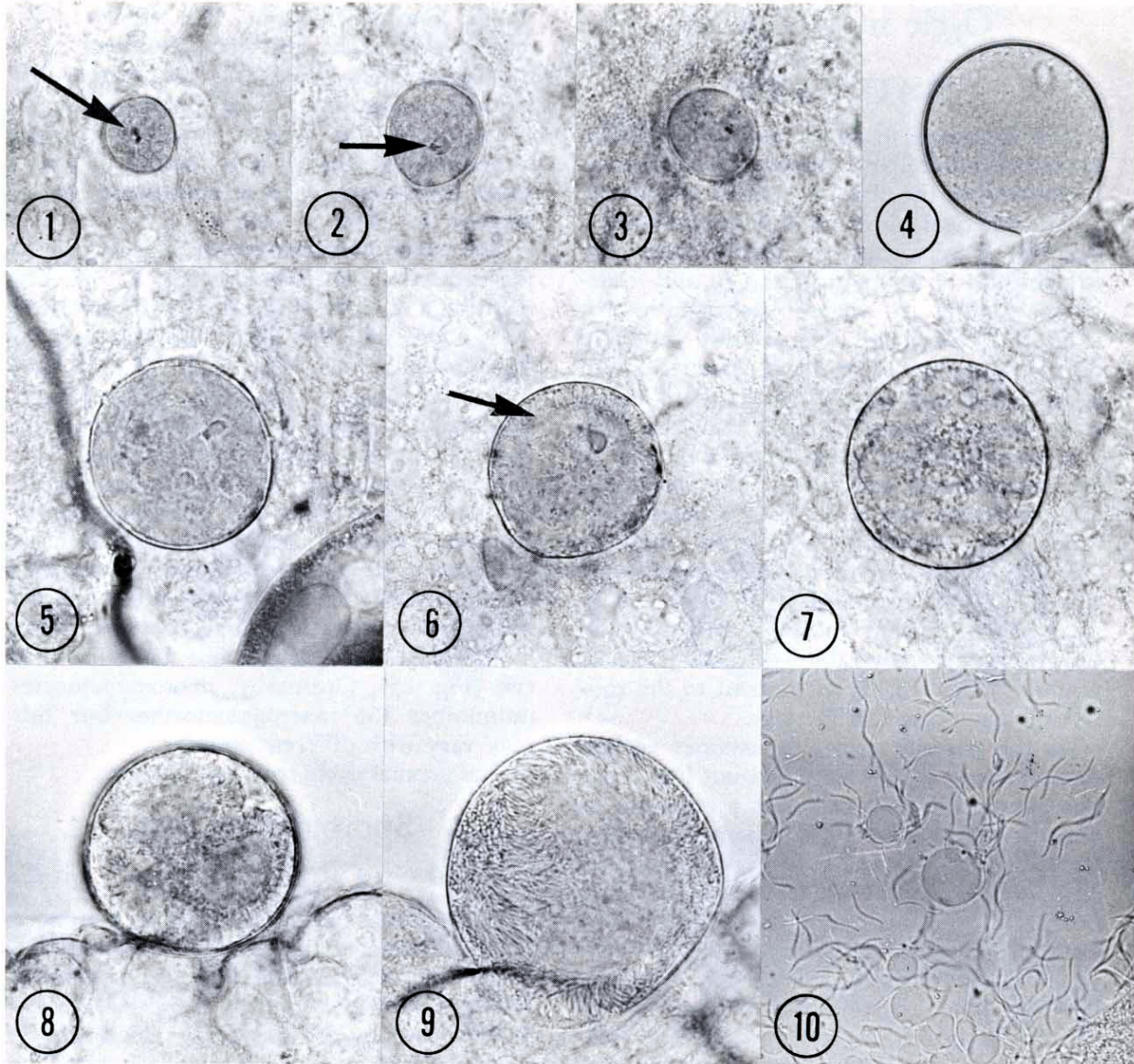


Plate XXXIV.—Developing oocysts and sporozoites of *Plasmodium malariae* in *Anopheles freeborni* mosquitoes. X 580.

Fig. 1. 10-day oocyst showing small clump of pigment.

Fig. 2. 11-day oocyst showing pigment clumped within small vacuole.

Fig. 3. 12-day oocyst.

Figs. 4, 5. 13-day oocysts.

Fig. 6. 14-day oocyst showing early differentiation.

Fig. 7. 14-day oocyst showing differentiation.

Fig. 8. 15-day oocyst.

Fig. 9. 17-day fully differentiated oocyst.

Fig. 10. Sporozoites present near salivary gland tissue 19 days after feeding.

9th day onwards, the pigment was clumped in a mass at the periphery. From the 3rd to the 9th day, daily increase in size of the oocyst was less than 2μ ; from the 9th day onward, the daily increase varied from 5 to 8μ . Oocysts ranged in size from 5 to 44μ . The minimum time required

for completion of the cycle was 15 days, the maximum, 21 days.

In our studies (Collins and Contacos, 1969), *A. freeborni* was the most suitable mosquito for studying the sporogonic cycle (Table 27). On day 6, at an incubation temperature of 25°C , the

mean oocyst diameter was 12 μ with a range of 9 to 14 μ . The oocysts continued to grow so that by day 14, the mean size was 38 μ with a range of 20 to 65 μ . The first signs of oocyst differentiation were apparent by day 14; sporozoites were present in the salivary glands on day 17.

A comparison of the oocyst growth rate of *P. malariae* with that of *P. cynomolgi* (Fig. 45) points-up the marked difference between the 2 parasites. The mean oocyst diameter of *P. cynomolgi* on day 8 is about the same as that of *P. malariae* on day 15. Sporozoites appear in the salivary glands 6 days sooner with *P. cynomolgi* than with *P. malariae*. The comparison of the sporogonic cycle of this parasite with *P. brasilianum* is presented elsewhere (Chapter 19).

Cycle in the Tissue

The first tissue stages of *Plasmodium malariae* were seen and described by Bray (1959, 1960). Liver biopsy specimens (8, 9, 10, 11, 12, and 12.5 days) were taken from 3 different chimpanzees which had received 72 to 110 salivary glands of *Anopheles Gambia*; 33 to 50 percent of them were infected with sporozoites of a Liberian strain of *P. malariae*.

TABLE 27.—Oocyst diameters of *Plasmodium malariae* in *Anopheles freeborni* mosquitoes.

Days post infection	<i>A. freeborni</i>		
	No.	Range	Mean*
6	104	9-14	12
7	105	11-19	14
8	185	12-21	17
9	169	14-27	20
10	146	13-32	21
11	268	13-45	27
12	218	14-51	29
13	195	19-59	37
14	260	20-65	38†
15	260	17-77	50†
16	348	19-88	48†
17	168	15-86	53†**
Totals	2426	9-88	

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

The 8-day tissue schizont was described as lying in the liver parenchyma cell usually, but not always, in a vacuole. The host cell was enlarged and the cytoplasm pushed aside in a crescent-shape around the parasite. The host cell nucleus was enlarged and pushed to one side. In over 50 percent of the parasitized parenchymal cells, 2 or more enlarged nuclei were present. The tissue schizont usually had a distinct

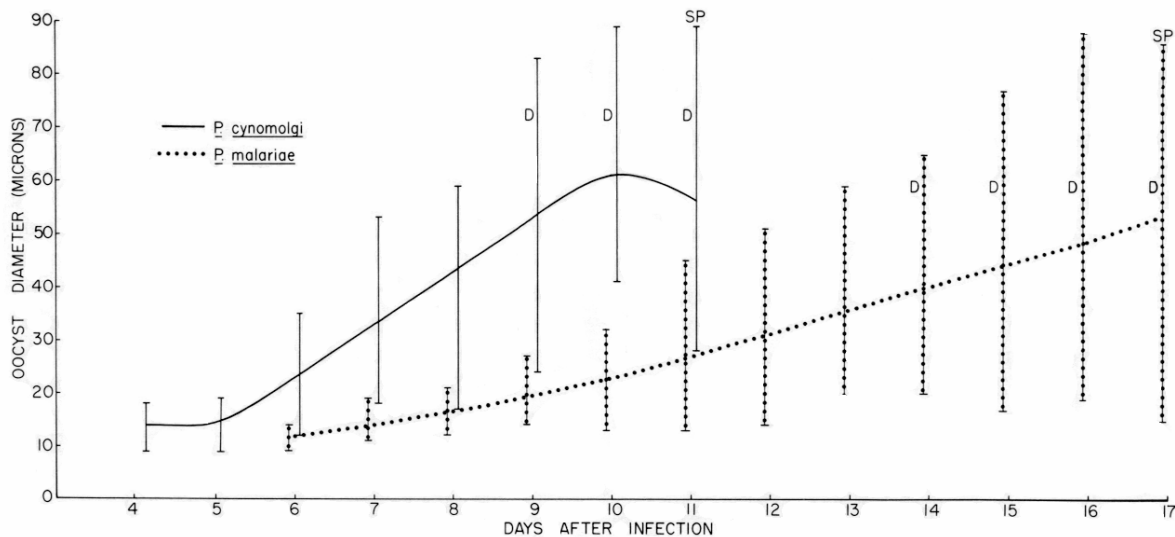


FIGURE 45.—Range in oocyst diameters and the mean oocyst diameter curves of *Plasmodium cynomolgi* and *P. malariae* in *Anopheles freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

envelope with no clefts. Its cytoplasm was generally homogeneous but was sometimes tenuous or in strands. Occasionally, the periphery showed tiny vacuoles. The nuclei were usually small dots measuring about 0.5 μ or round compact masses of chromatin measuring about 1 μ in diameter. The average size of the 8-day tissue schizont was 12.5 μ (range of 9.5 to 17 μ). The average number of nuclei was 25 (range of 7 to 49).

The 9-day schizonts were generally like the 8-day forms except for the scatter in size, the range was 8.5 to 21 μ (average 12.7 μ). The average number of nuclei was 27 (range of 10 to 83).

The 10-day schizonts were usually within a vacuole in the parenchyma cell. The parasite had pushed the enlarged nucleus, or nuclei, of the enlarged host cell, to one side. The cytoplasm was homogeneous and reasonably dense. In about half of the parasites, the cytoplasm was pulled slightly away from the distinct envelope; in about 40 percent, it had not contracted away from the envelope; in the remaining 10 percent, the cytoplasm was irregularly vacuolated. Some showed, what was described as, 3- or 4-point type nuclei. The size of the 10-day stage averaged 22.1 μ (range of 15 to 29 μ); the average number of nuclei was 100 (range of 33 to 201).

The 11-day schizonts were smaller than the 8- or 9-day stages, the average being 11.7 μ (range of 9.5 to 14 μ); the average number of nuclei was approximately 15 (range of 6 to 30). The 12-day tissue parasite had a distinct envelope with dense cytoplasm in the central portions of the parasite. The periphery consisted of vacuoles measuring 3 to 6 μ in diameter. The cytoplasm contained a number of small basophilic aggregates. The schizont was sometimes slightly lobulated. The average size was 41 μ (range of 34 to 47 μ). The average number of nuclei was 712 (range of 435 to 1151). The 12.5-day schizonts resembled the 12-day forms except for 2 characteristics; namely, the parasite was markedly lobulated and the peripheral vacuolations were more marked--large peripheral vacuoles measuring 5 to 8 μ were commonly observed.

Bray mentioned several general observations concerning the tissue schizonts at

all ages; namely, the nuclei were always randomly distributed, there were no pseudocytomeres, no evidence of septal formation, or plasmotomy. He saw no mature schizonts or any evidence of sporulation. Bray's studies showed quite distinctly that the tissue stages of *P. malariae* in the chimpanzee differ markedly from the other species of human malaria. It differs from vivax and falciparum malaria in that it causes enlargement of the host cell nucleus, the older forms show considerable vacuolation of the peripheral cytoplasm, it produces fewer merozoites, and the growth rate is considerably slower. It differs from *P. ovale* in that the nuclei of malariae tissue stages are smaller, fewer in number, and its growth rate is slightly slower. The first evidence of parasitemia in the chimpanzees was seen 16 days after the inoculation of sporozoites.

Lupascu *et al* (1967) determined the "exact duration" of the "prepatent period" of the VS (Roumanian) strain of *P. malariae* by the subinoculation of blood into new patients. Only the recipients given blood 15 days or more after inoculation of sporozoites became infected. In other words, with this strain of *P. malariae*, the exoerythrocytic cycle requires exactly 15 days and, therefore, the prepatent period could be as early as day 15.

Lupascu *et al* also described the later tissue stages of *P. malariae*. They inoculated a 3 year-old chimpanzee with a total of 110 salivary glands from *Anopheles labranchiae atroparvus* mosquitoes, 86 to 100 percent infected with the VS strain. Liver biopsy material was obtained at 12, 13, 14, and 15 days. All 4 biopsy specimens contained tissue schizonts. The 12-day stages were usually oval, and, less often, spherical in shape. The contour was usually smooth but occasionally disturbed by the presence of peripheral vacuoles. The parasitized perenchymal cells were greatly enlarged. The host cell nucleus was pushed to one side and much enlarged, sometimes twice its normal size, and frequently pyknotic. The mean diameter of the 12-day schizont was 30 μ . The nuclei were round and interspersed throughout the cytoplasm, being somewhat scantier towards the margin of the parasite. The diameter of the nuclei was approximately 1.5 μ . The cytoplasm tended to accumulate either as individual

flocculi or as larger masses. Numerous vacuoles of all sizes were present. The largest vacuoles were 10 μ . The parasite had a definite membrane.

The 13-day forms had an average size of 44 μ , indicating considerable growth in 24 hours. This rapid growth probably accounted for the pronounced lobulation. The internal structure of the parasite was much the same as that of the 12-day schizont except that the nuclei were "less loosely packed." Cytoplasmic clumps and vacuoles persisted and clefts were usually seen.

The 14-day schizonts reached a maximum mean diameter of 47 μ . They were more difficult to measure because of the lobulated surface. The most striking feature was the extensive cleavage of the cytoplasm in some of the schizonts. The cytoplasm was condensed into long strands, studded with nuclei, and sometimes smaller clefts were present throughout the parasite. The nuclei were "tightly packed" and measured about 2 μ in diameter; vacuoles were obvious. The limiting membrane of the parasite was distinct and occasionally thrown into pronounced folds. Only the 14-day schizonts exhibited merozoites.

The 15-day schizonts were considered mature and remnants of ruptured schizonts were readily observed. The mature forms had a mean diameter of about 51 μ . Their shape was mostly oval, although irregular processes (lobulations) were observed. The nuclei, of the near-mature schizonts, lost their spherical shape just prior to the final division, became triangular, and measured as much as 3.3 μ in their greatest dimension. The cytoplasm formed patterns although the nuclei did not enter this material and no cytomeres or any form of aposchizogony was observed. Large vacuoles were present but were less common than in the nearly-mature stage. The authors described curious reddish strands in the cytoplasm of some of the schizonts and considered this feature unique among tissue forms of malaria parasites. The mature schizont, except for the strands and a few vacuoles, consisted, almost entirely, of merozoites within the outer limiting membrane of the parasite. The largest mature schizont measured 41 by 85 μ , the average was 56 μ . The number of merozoites was directly dependent upon the size of the mature schizont. The largest

number of merozoites was estimated to be 18,650 and the smallest 7,500. The merozoite was a sphere measuring 2 μ in diameter, consisting of cytoplasm which indents the nucleus to render it characteristically crescentic in shape.

Lupascu *et al* (1967) in their summary gave the main characteristics of the tissue schizonts of the VS strain of *P. malariae* as: enlargement of the host cell nucleus, many peripheral and internal vacuoles, no cytomeres, large clefts, red-staining strands, and plaques in the mature schizonts.

Course of Infection

As mentioned earlier, the shortest prepatent period for *P. malariae* could be as early as 15 days, on the basis of the findings of Lupascu's subinoculation experiments. However, in actuality, the earliest reported prepatent period has been 16 days in a West African strain (Shute and Maryon, 1951).

Generally, the prepatent periods, with this species, have been longer. Boyd and Stratman-Thomas (1933) transmitted 2 strains in which the prepatent periods were 27, 32, and 37 days. Mer (1933) transmitted a Palestinian strain to 3 patients; prepatent periods were 26, 28, and 31 days. Prepatent periods of 23 to 26, days were reported by de Buck (1935) for 4 patients infected with a Vienna strain. Boyd and Stratman-Thomas (1936) reported a prepatent period of 37 days (thin smear examination); the incubation period was 40 days. This patient had only 5 paroxysms with 4 chills. The paroxysms subsided spontaneously although parasites were found as late as 173 days after exposure to infection. They also reported a prepatent period of 28 days and an incubation period of 30 days in another patient. Marotta and Sandicchi (1939) reported incubation periods of 23 and 29 days in 2 patients.

Boyd (1940) reported on 3 different strains: USPHS, Jones, and Weaver. The prepatent periods ranged from 28 to 37 days (median of 34 days) and the incubation periods from 30 to 49 days (median of 36 days). Siddons (1944) transmitted an Indian strain of *P. malariae* to a

patient who exhibited a prepatent period of 30 days and an incubation period of 36 days. Young and Burgess (1947) observed prepatent periods of 29 and 59 days in 2 patients whose incubation periods were 28 and 69 days. These authors considered the prepatent period of 59 days to be due, possibly, to some immunity on the part of the patient which was substantiated by a short duration of parasitemia and extremely low parasite counts. Mackerras and Ercole (1948) reported a prepatent period of 24 days for a Melanesian strain. Kitchen (1949) reported mean prepatent periods of 32.2 days (range 27 to 37 days) and mean incubation periods of 34.8 days (range 29 to 40 days) for naturally induced infections of American strains of *P. malariae*.

Young and Burgess (1961) transmitted the USPHS strain of *P. malariae* to 2 patients and observed prepatent periods of 33 and 36 days and incubation periods of 28 and 43 days. Ciuca *et al* (1964) reported prepatent periods ranging from 18 to 25 days for a local Roumanian strain, now known as the VS strain. Lupascu *et al* (1968) reported incubation periods of 18 to 19 days with the VS strain of *P. malariae*. As these data show, there is a wide range in the length of the prepatent periods in naturally transmitted *P. malariae* (18 to 59 days) and an equally wide range in the incubation periods (28 to 69 days).

In our transmission studies with a Nigerian strain of *P. malariae*, involving 4 volunteers, we have observed prepatent periods ranging from 24 to 33 days (Contacos and Collins, 1969); with the VS strain, in 4 patients, the pre-patent periods ranged from 21 to 30 days.

Malariae malaria infections are considered to be relatively benign when compared to falciparum malaria but more severe than vivax infections. Probably its most peculiar characteristic is its pronounced chronicity, especially the unusually long period of time during which parasites can remain 'dormant' within the host. A less constant characteristic is the tendency to cause renal damage which was dealt with in some detail by Giglioli (1930).

The onset of *P. malariae* attacks is generally more gradual than observed with many vivax and falciparum infections. The initial remittent fever pattern characteristic of vivax infections is seen less frequently in malariae infections. Rather, they exhibit an intermittent

pattern (Kitchen, 1949) because the sporulation of this species is highly synchronous.

Young *et al* (1940) studied the periodic phenomenon of the asexual cycle of quartan malaria in Negro paretics. They found that their strain of malariae malaria (USPHS) exhibited a high degree of synchronicity, the asexual erythrocytic cycle repeating itself every 72 hours, but being more exact in some patients than in others. The length of time consumed by the different growth stages was: 54.2 hours for the trophozoites, 10.4 hours for the young schizonts, and 7.4 hours for the segmenters or late schizonts. The process of segmentation required roughly 6 hours. The rise in temperature closely followed the progress of segmentation and reached its height approximately at the end of the process.

Young *et al* (1940a) showed that modifying the external conditions of the host affected the time of sporulation of *P. malariae* in man when patients were placed under reversed conditions of activity; the segmenter-number peaks in one changed from the normal 9:00 a.m. hour to 9:00 p.m. In the other, the cycle was shortened until the segmenters peaked about 22 hours before normally expected. Two other patients were placed under reversed conditions, except lighted continuously. In these, the segmenter-peak time changed from 9:00 a.m. to 9:00 p.m. When one of them was returned to a normal schedule, the segmenter-number peaks returned to the normal time, 9:00 a.m.

Young *et al* (1941) presented data on 420 paroxysms occurring in 15 patients infected with the USPHS strain of *P. malariae*. Chills were observed 102 times (24 percent). Some patients experienced chills more often than others; one had 9 chills in 14 paroxysms (64 percent), while another had only 1 chill in 45 paroxysms (2 percent). Temperatures at the beginning of chills were found to range from 97.6° F to 106.0° F rectally, with an average of 101.8° F. Temperatures at the end of the chills ranged from 97.8° F to 106.0° F, with an average of 103.5° F. The greatest increase in temperature during a chill was observed in a patient whose temperature rose from 98.0° F to 103.0° F during a chill lasting 45 minutes. Two patients experienced the opposite; in other words, a drop in temperature during a chill. One had a

temperature of 106.0° F at the beginning and 105.0° F at the end of the chill which lasted 50 minutes. Another patient dropped from 104.2° F at the beginning of the chill to 103.2° F at the end of the chill which lasted 15 minutes. It is interesting that in both these cases, very high temperatures had already been reached by the time the chill began. The durations of all chills ranged from 13 to 195 minutes with an average of 53 minutes (Fig. 46).

According to Kitchen (1949), the malariae paroxysm is less often introduced by chills than are vivax paroxysms. Boyd (1940) found that the initiation of the malariae paroxysm by chills was extremely variable. Indeed, one of 5 naturally induced cases studied by him had no chills at all. In Kitchen's observations, on patients infected with the Trinidad strain, the temperature at the onset of the chill varied between 97.4°F and 104.4°F (mean of 100.3° F). The mean duration of the chills was 55.97 minutes. The mean temperature peak was 104.98° F reached, on an average, 2 hours, 54 minutes after the onset. The interval from peak temperature until the temperature returned to normal was 10 hours, 23 minutes.

The entire paroxysm, therefore, occupied a period of 13 hours and 17 minutes. *Plasmodium*

malariae paroxysms are longer in duration than those of *P. vivax*.

In the Young *et al* series, the average fever peak for the 420 paroxysms was 104.1°F rectally, with the highest temperature recorded, 106.4° F. The duration of the fevers ranged from 5 to 32 hours with an average of 10 hours, 58 minutes. The average time for the interval from the beginning of fever to the fever peak was 4 hours, 45 minutes and from the fever peak to the end of the fever was 6 hours, 13 minutes. These authors also observed that some paroxysms were introduced by a chill, while others were devoid of chills. When a chill accompanied the paroxysm, the fever was significantly higher (104.6° F as compared to 104.0° F without a chill) and the duration of the fever was 1 hour, 16 minutes shorter (10 hours duration for the paroxysms with chills as compared to 11 hours, 16 minutes for the paroxysms without chills). In addition, it was obvious that this shortening of the fever period occurred almost entirely in the period between the onset of the paroxysm and the peak of the fever. They believed that the chill exerted a definite influence on the character of the paroxysm. When a chill occurred, the duration of the fever was shorter (1 hour, 16 minutes) which was due, apparently, to the

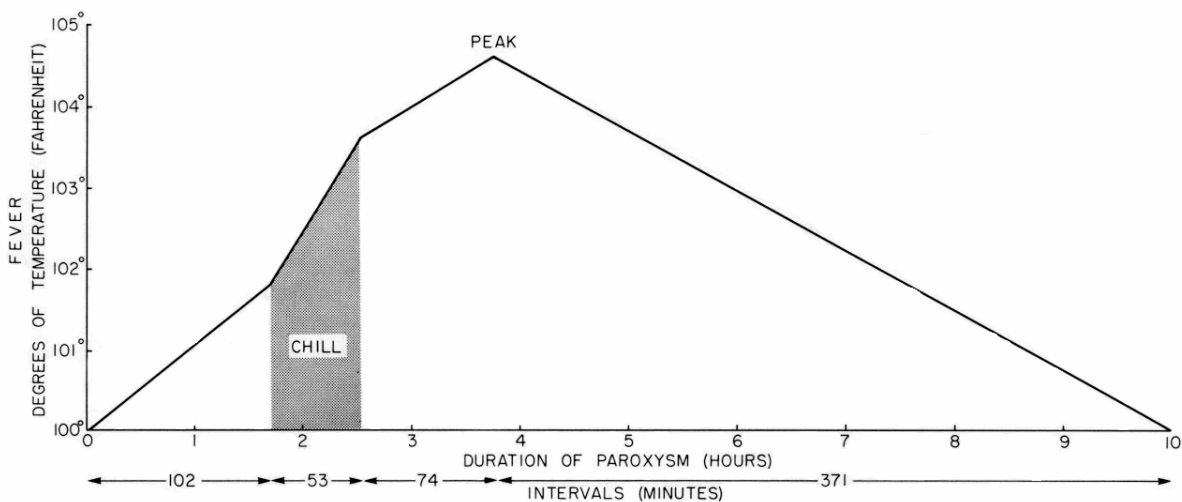


FIGURE 46.—The temperature curve in relation to the chill in 102 paroxysms in *Plasmodium malariae* infections (after Young, Coatney, and McLendon, 1941).

shortening of the period when the temperature was rising, and, that the maximal temperature was significantly higher than in paroxysms without chills. The greatest proportionate increase in temperature occurred during the chilling period (Fig. 46).

Generally, the degree of parasitemia is lower for *P. malariae* than for either vivax or falciparum malaria. Boyd (1940) reported mean parasite densities ranging up to 12,500 per mm^3 . However, there was one patient in his series, whose infection resulted in a fatal outcome, in whom the count exceeded 100,000 parasites per mm^3 . Young and Burgess (1947) observed parasites at least through 58 days in a patient having a maximum parasite count of 3,280 per mm^3 of blood. Mackerras and Ercole (1948) did not observe parasitemias which exceeded 2,300 per mm^3 .

Kitchen (1949) stated that the parasitemias observed in his *P. malariae* infections were comparatively low. Counts above 20,000 per mm^3 were unusual and counts above 50,000 per mm^3 were rare. He indicated that most patients do not attain counts above 10,000 per mm^3 during their entire attack. After the first few weeks, the counts tend to drop and remain below 5,000 per mm^3 . Young and Burgess (1961) reported parasitemias up to 17,580 parasites per mm^3 , with an average of 3,799 per mm^3 in symptomatic cases and up to 4,920 per mm^3 , with an average of 999 per mm^3 in

asymptomatic cases. Ciuca *et al* (1964) reported maximum parasitemias of 6,700 and 8,852 per mm^3 for sporozoite- and parasitized blood-induced infections, respectively. In our studies with a Nigerian strain of *P. malariae*, a maximum parasite count of 11,200 per mm^3 has been observed in a blood-induced infection (Collins and Contacos, 1969).

Figure 47 shows the median parasitemia curves for Caucasian and Negro patients infected with the USPHS strain of *Plasmodium malariae*, as well as curves for the minimum and maximum parasitemia. It can be readily seen that the median parasitemia curves for the Caucasian and Negro patients show only minor differences. Median peak parasitemia occurs some time between day 15 and day 20. The parasitemia subsequently persists at an almost constant level, tapering off very gradually during the next 40 days, in the Negro patients and only a very little, if at all, in the Caucasians. The median maximum parasitemias were approximately 5,000 per mm^3 at day 25 and 6,000 at day 28 for the Negro and Caucasian patients, respectively. The maximum parasitemia attained by a Caucasian patient was 22,000 parasites per mm^3 of blood at day 17, and, for Negro patients, 25,000 per mm^3 at day 18, and 26,500 per mm^3 at day 40. There were slight differences between the parasitemias in the Caucasian and Negro patients from day 30

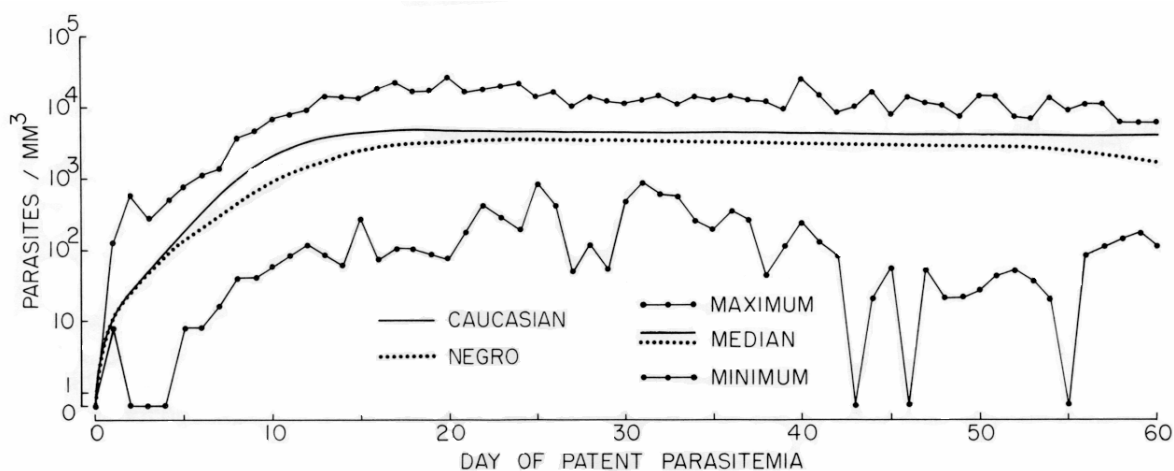


FIGURE 47.—Minimum and maximum parasitemia curves in 54 human volunteers infected with *Plasmodium malariae* and median parasitemia curves for 29 Caucasian and 25 Negro volunteers, all infected by the inoculation of parasitized blood.

on. By day 30, the median parasite count for the Caucasian patients was approximately 5,000 per mms whereas for the Negro patients, it was approximately 3,000 per mm³. Between day 30 and 50, the median parasite counts for the Caucasian patients ranged between 3,000 and 6,000 per mm³ whereas for the Negro patients, it was between 2,000 and 3,500 per mm³. After day 50, the median parasite counts for the Caucasian patients ranged between 3,000 and 4,000 per mm³ whereas in the Negro patients, the counts ranged between 1,000 and 3,000 per mm³. In other words, there appeared to be a slightly earlier trend toward chronicity in the Negro patients than in the Caucasian patients infected with the same strain.

The duration of *P. malariae* infections can be extremely long, especially in the form of subpatent parasitemias. Boyd and Stratman-Thomas (1936) observed malariae parasites as late as 173 days after exposure to infection. Boyd (1940) found that in infections induced by mosquito bites, *P. malariae* attacks, including recurrences, had a mean duration of 170 days in Caucasians and 76 days in Negroes. The mean duration of all naturally induced clinical attacks, regardless of race, was 132 days. Boyd (1947) reported that a naturally induced infection with *P. malariae* had persisted in a latent-chronic state for a period of 4,305 days (11 years, 9 months, 14 days); this was established when the patient, who had received no specific therapy, gave 250 ml of blood for a transfusion. Shute and Maryon (1955) reported "true relapse" of *P. malariae* infection in 2 patients 12 and 32 years after infection. What they reported, probably, were recrudescences since it is not clear whether the therapy given was adequate to totally eliminate all the blood stages. Ciuca *et al* (1956) published on a case in which parasites were observed after 18 years. Lentini and Tecce (1955) observed a patient whose infection relapsed (recrudesced) 45 years after leaving an endemic area. Guazzi and Grazi (1963) reported an infection which relapsed, subsequent to a splenectomy, approximately 53 years after the primary attack. Diamond (1966) found *P. malariae* parasites in a blood film from a patient who had immigrated from Antigua to England 12 years earlier and had no previous history of malaria.

Finally, a comment about true relapse activity in *P. malariae*. Ciuca *et al* (1964) did not observe relapse activity in any of their patients whose infections were induced by bites of infected mosquitoes. They concluded, therefore, that there was no secondary exoerythrocytic cycle. In this connection, we also have observed no relapse activity after adequate blood schizonticidal therapy in our volunteers exposed to infection with a Nigerian strain of *P. malariae*. It seems safe to say that, in this respect, *P. malariae* resembles *P. falciparum* more than it does the human tertian malarias which do relapse.

Plasmodium malariae can infect the chimpanzee (Rodhain, 1948; Garnham *et al*, 1956). Rodhain observed maximum parasitemias of 7,000 per mm³. Garnham *et al* observed a maximum parasitemia of 160,000 per mm³ in a splenectomized chimpanzee. Bray (1960) observed parasitemia of *P. malariae* in splenectomized chimpanzees, between 25,000 and 50,000 parasites per mm³ of blood. Some consider *P. malariae* of man and *P. rodhaini* in the chimpanzee to be one and the same species. Maybe they are one and the same but, in our opinion, adequate proof is lacking.

The first adaptation of *Plasmodium malariae* to *Aotus trivirgatus* monkeys was reported by Geiman and Siddiqui (1969). One hundred and thirty days after inoculation, the parasitemia reached a peak--of 97,920 per mm³ of blood. During the succeeding one year of observation, the parasitemia fluctuated between 200 and 26,000 per mm³. The infection was subsequently passaged to splenectomized and to intact *A. trivirgatus* monkeys; their parasitemias ranged from 100 to 23,000 per mm³.

In our own studies (Contacos and Collins, 1969; Collins and Contacos, 1969), we have been able to transfer the infection from man to splenectomized *A. trivirgatus* monkeys on 2 occasions (Fig. 48). The parasitemia was slow to rise and reached a mean parasite level of approximately 1,000 per mm³ on the 25th day of patent parasitemia. Subsequent passage to 10 other splenectomized monkeys resulted in a peak median parasitemia of approximately 2,500 per mm³ by day 15. Thereafter, the parasitemia was maintained at approximately the same level as that in the primary passages.

Plasmodium malariae grows well in this monkey as indicated by the nearly normal appearance of the parasites in the blood, (Plate XXXV) the long persistence of the infection, and its ready infectivity to mosquitoes (Fig. 49). In one monkey (AO-74), *A. freeborni* mosquitoes were allowed to feed beginning day 11 and through day 108 of parasitemia (no feedings were made on days 48 through 56). Infections were obtained on 74 of the 89 feeding days. Although, in general, the percentage of infection was low, there were indications, at least, of an every-third-day peak in the mosquito infection rate during certain periods, such as, on days 16, 19, 25, 28, 34, 37, 40, 43, and 46. Although not as pronounced as with some of the tertian malarias (see Chapters 6 and 23), it suggests that infectivity may be correlated with the schizogonic cycle in the vertebrate host.

Experimental transmission of *P. malariae* by the bites of infected mosquitoes has been demonstrated by a number of workers as listed in Table 28. To that list we can add our experiences in which a Nigerian strain of *P.*

malariae has been transmitted to 6 volunteers by the bites of infected *A. freeborni* mosquitoes and, the Roumanian VS strain, to 4 volunteers via the bites of *A. atroparvus*.

Host Specificity

Plasmodium malariae is found naturally in man and, possibly, in chimpanzees. Experimentally, infections have been obtained in owl monkeys, *Aotus trivirgatus*, although, to date, only blood induced infections have been reported.

Mosquitoes which have been reported as susceptible to infection with *P. malariae* are listed in Table 28. In addition, we have obtained infections in *A. b. balabacensis* mosquitoes. A comparative infectivity study with a strain of this parasite from Nigeria (Table 29), indicates that *A. freeborni* was the most susceptible followed by *A. b. balabacensis*, *A. stephensi*, *A. atroparvus*, and finally, *A. maculatus*.

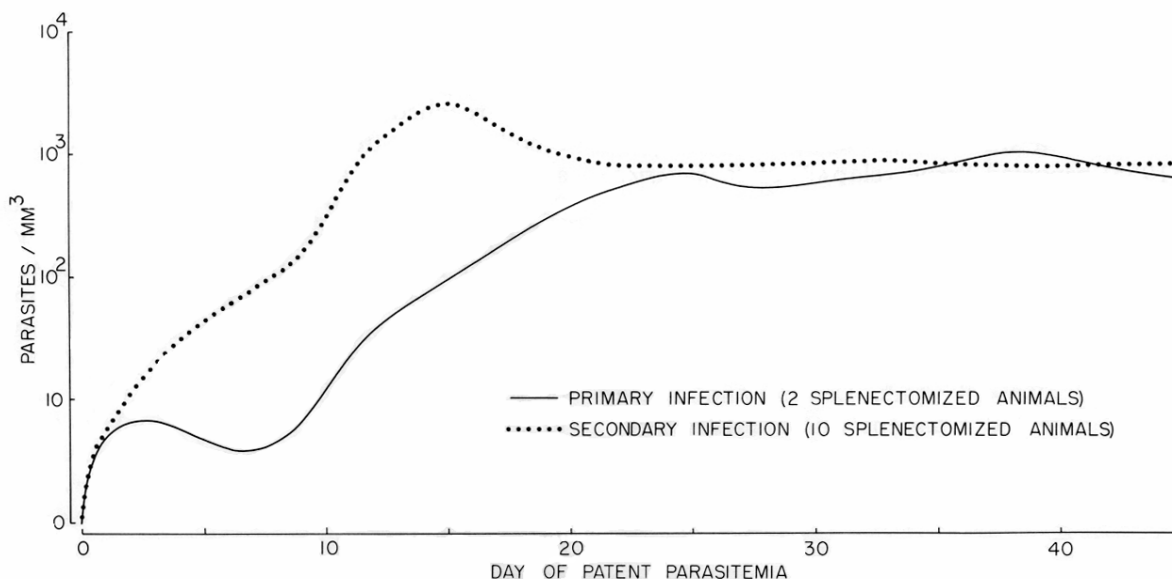
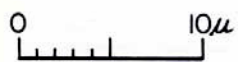
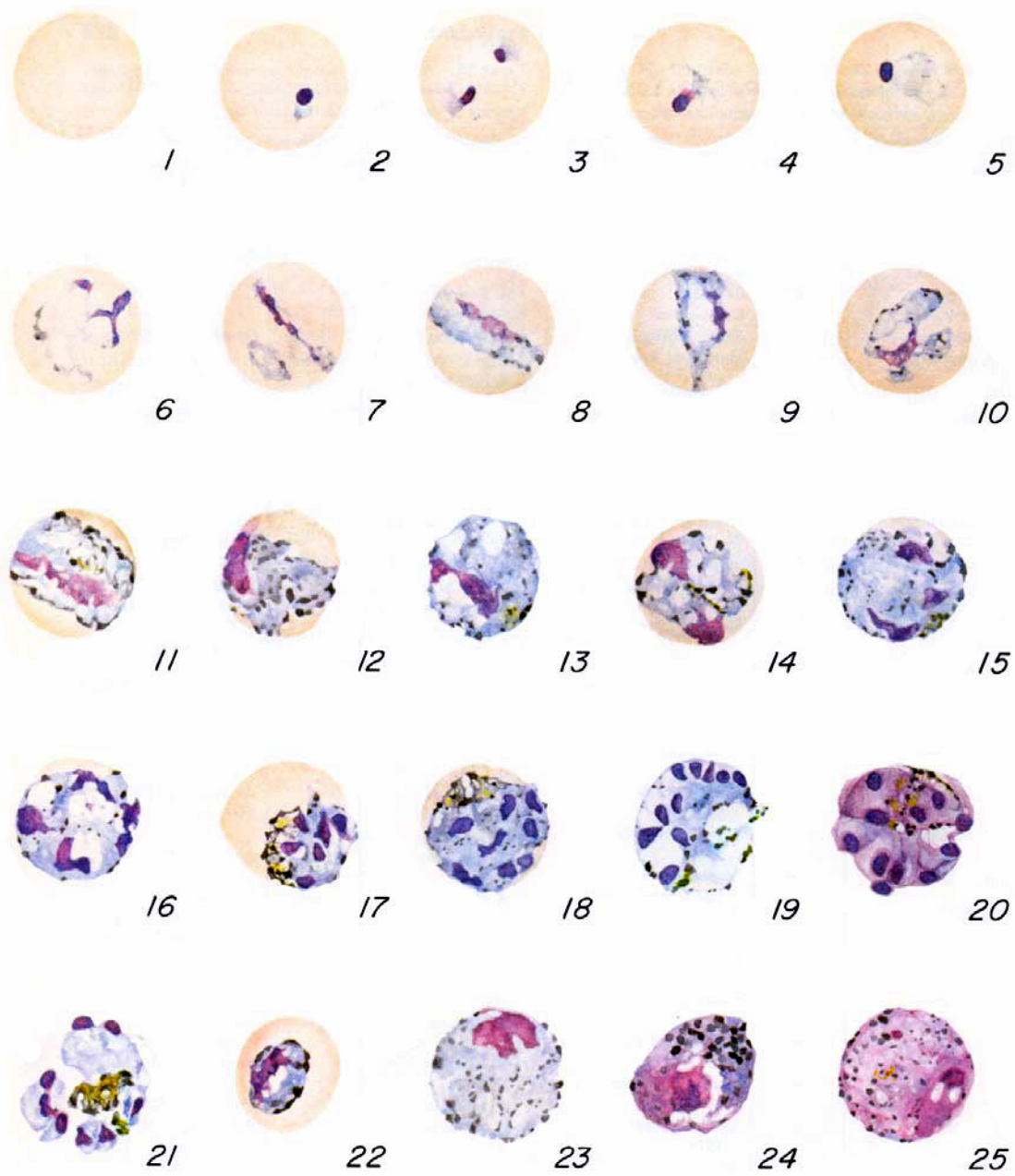


FIGURE 48.—Median parasitemia curves of *Plasmodium malariae* in *Aotus trivirgatus* monkeys after primary inoculation of parasitized blood from man and secondary inoculation into additional monkeys.

TABLE 28.—Anopheline mosquitoes tested for susceptibility to infection with *Plasmodium malariae* and those which effected transmission.

Mosquito species	Transmission	References	Mosquito species	Transmission	References
<i>Anopheles acoutius</i>	--	Schuurman and Huinink, 1929	<i>A. melas</i>	--	Garnham, 1966.
<i>A. annulipes</i>	--	Garnham, 1966*	<i>A. messeae</i>	+	Marotta and Sandicchi, 1939
<i>A. atroparvus</i>	--	Jancso, 1921	<i>A. minimus</i>	--	Anazawa, 1931
<i>A. atroparvus</i>	--	James, 1931	<i>A. moucheti</i>	--	Garnham, 1966
<i>A. atroparvus</i>	+	de Buck, 1935	<i>A. plumbeus</i>	--	Gendel'man, 1936
<i>A. atroparvus</i>	--	Cambournac, 1942	<i>A. punctulatus</i>	+	Mackerras and Roberts, 1947
<i>A. atroparvus</i>	--	Shute, 1951	<i>A. punctulatus</i>	--	Mackerras and Ercole, 1948
<i>A. atroparvus</i>	+	Shute and Maryon, 1955	<i>A. punctipennis</i>	--	Mayne, 1932
<i>A. atroparvus</i>	+	Ciuca <i>et al</i> , 1964	<i>A. punctipennis</i>	--	Young and Burgess, 1961
<i>A. atroparvus</i>	--	Constantinescu and Negulici, 1967	<i>A. quadrimaculatus</i>	+	Boyd and Stratman-Thomas, 1933
<i>A. aztecus</i>	--	Young and Burgess, 1961	<i>A. quadrimaculatus</i>	--	Boyd and Stratman-Thomas, 1934
<i>A. claviger</i>	--	Grassi <i>et al</i> , 1899	<i>A. quadrimaculatus</i>	+	Boyd and Stratman-Thomas, 1936
<i>A. culicifacies</i>	--	Stephens and Christophers, 1902	<i>A. quadrimaculatus</i>	+	Young and Burgess, 1947
<i>A. culicifacies</i>	--	Iyengar, 1933	<i>A. quadrimaculatus</i>	--	Young <i>et al</i> , 1948
<i>A. culicifacies</i>	--	Siddons, 1944	<i>A. quadrimaculatus</i>	+	Young and Burgess, 1961
<i>A. darlingi</i>	--	Garnham, 1966*	<i>A. sacharovi</i>	--	Khodukin and Lisova, 1930
<i>A. fluviatilis</i>	--	Garnham, 1966*	<i>A. sacharovi</i>	+	Averbouch and Mer, 1933
<i>A. freeborni</i>	+	Young and Burgess, 1947	<i>A. splendidus</i>	--	Anazawa, 1931
<i>A. freeborni</i>	--	Young and Burgess, 1961	<i>A. stephensi</i>	--	Sur <i>et al</i> , 1932
<i>A. freeborni</i>	+	Contacos and Collins, 1969	<i>A. stephensi</i>	--	Iyengar, 1933
<i>A. fuliginosus</i>	--	Stephens and Christophers, 1902	<i>A. stephensi</i>	--	Russell and Mohan, 1939
<i>A. fuliginosus</i>	--	Anazawa, 1931	<i>A. stephensi</i>	--	Knowles and Basu, 1943
<i>A. fuliginosus</i>	--	Sur <i>et al</i> , 1932	<i>A. stephensi</i>	+	Shute, 1951; Shute and Maryon, 1951
<i>A. fuliginosus</i>	--	Basu, 1943	<i>A. sundaicus</i>	--	Swellengrebel <i>et al</i> , 1919
<i>A. funestus</i>	--	Garnham, 1966 *	<i>A. sundaicus</i>	--	Hylkema, 1920
<i>A. gambiae</i>	--	Gordon and Davey, 1932	<i>A. sundaicus</i>	--	Anazawa, 1931
<i>A. gambiae</i>	--	Muirhead-Thompson, 1957	<i>A. sundaicus</i>	--	Iyengar, 1933
<i>A. hyrcanus sinensis</i>	--	Swellengrebel <i>et al</i> , 1919	<i>A. sundaicus</i>	+	Brumpt, 1949
<i>A. hyrcanus sinensis</i>	--	Anazawa, 1931	<i>A. tessellatus</i>	--	Anazawa, 1931
<i>A. hyrcanus sinensis</i>	--	Khaw and Kan, 1934	<i>A. varuna</i>	--	Sur <i>et al</i> , 1932
<i>A. jeyporiensis</i>	--	Garnham, 1966 *	<i>A. varuna</i>	--	Iyengar, 1933
<i>A. lindesayi</i>	--	Anazawa, 1931			
<i>A. maculatus</i>	--	Green, 1929			
<i>A. maculatus</i>	--	Anazawa, 1931			
<i>A. maculatus</i>	--	Strickland <i>et al</i> , 1940			

*Listed by Garnham as experimental hosts; reference not given.



H. H. Nicholson

PLASMODIUM MALARIAE IN AOTUS TRIVIRGATUS

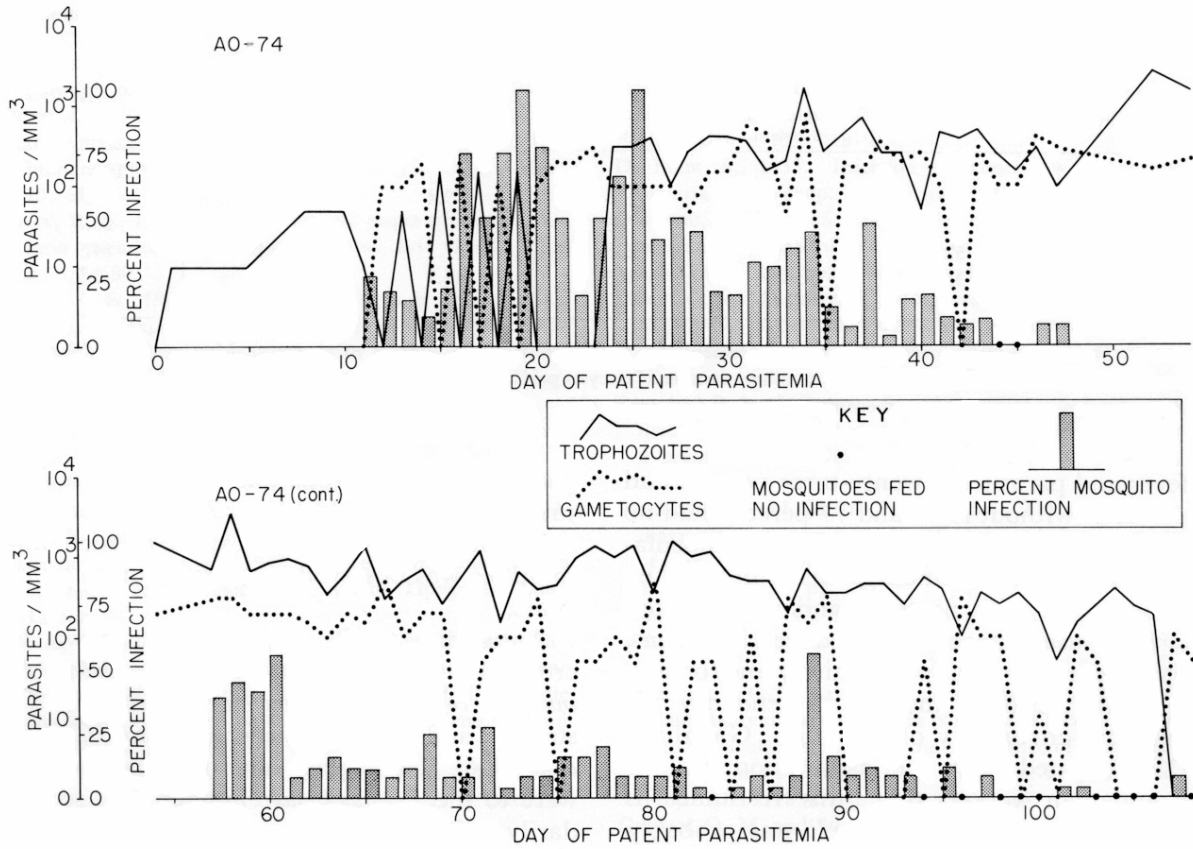


FIGURE 49.—Course of parasitemia and infectivity to *Anopheles freeborni* mosquitoes of an infection of *Plasmodium malariae* in an *Aotus trivirgatus* monkey (AO-74).

Immunity and Antigenic Relationships

Natural infections of *Plasmodium malariae* give rise to a strong immunity but apparently not as strong as those resulting from repeated blood-induced infections. Ciuca *et al* (1934) showed that many patients convalescing from blood-induced quartan malaria acquire a sterile immunity (i.e., large volumes of blood from

them were not infective). They also reported, that in patients reinoculated repeatedly with the same strain, the percentage of takes decreased. They showed, also, that homologous immunity was not only clinical but also parasitologic. Reinoculations with the same strain of parasite, after cessation of clinical manifestations, resulted in varying degrees of immunity to clinical symptoms and to parasitemia. By the 5th reinoculation, no patients showed fever and

PLATE XXXV.—*Plasmodium malariae* in the owl monkey, *Aotus trivirgatus*.

- Fig. 1. Normal red cell.
- Figs. 2-5. Young trophozoites.
- Figs. 6-10. Growing trophozoites, note double infection (Fig. 6).
- Fig. 11-13. Nearly mature and mature trophozoites.

- Figs. 14-20. Developing schizonts.
- Fig. 21. Mature schizonts.
- Figs. 22, 23. Developing and mature macrogametocytes.
- Figs. 24, 25. Developing and mature microgametocytes.

TABLE 29.—Comparative infectivity of *Plasmodium malariae* to *Anopheles freeborni*, *A. b. balabacensis*, *A. stephensi*, *A. atroparvus*, and *A. maculatus*.

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
F-1						100
F-1 : Bal	13	223	116	9.4	17.2	80.4
F-1 : St-1	5	134	65	9.7	4.6	20.3
F-1 : Atro	52	996	238	37.1	12.2	14.9
F-1 : Mac	33	554	865	43.7	11.3	3.5

* F-1 = *Anopheles freeborni*, Bal = *A. b. balabacensis*, St-1 = *A. stephensi*, Atro = *A. atroparvus*, Mac = *A. maculatus*.

** 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.

parasites. Twenty-three percent had parasites without fever and 77 percent had no fever and no parasites. By the 7th reinoculation, homologous immunity was complete, i.e., 100 percent with no fever and no parasites.

In naturally acquired or induced infections, parasites rarely disappear entirely and/or permanently; rather, there is a tendency for extreme chronicity of infection. Garnham (1966) stated that the assumption by the Roumanian workers that "this 'resistance' is of the nature of premunition" was probably correct. Ciuca *et al* (1955) reported that the forms of immunity acquired as a result of *P. malariae* infection present characteristics entirely different from those for *P. vivax* and *P. falciparum* in the sense that the proportion of residual immunity (immunity without premunition) is superior to that of "concomitante" immunity (immunity with premunition), being, respectively, 72.8 and 27.2 percent.

Plasmodium malariae offers better antigenic quality than the other human malaras for stimulating a more durable immunity which maintains itself even after the disappearance of parasites. Homologous and heterologous

immunity were considered by Ciuca *et al* to develop in parallel. The duration of such immunities was observed to persist from 5 to 14 years.

Antibodies to *P. malariae* were shown to cross-react to *Plasmodium fieldi*, *P. gonderi*, *P. inui*, *P. coatneyi*, *P. knowlesi*, *P. cynomolgi* (B strain), and *P. brasilianum* antigens (Collins *et al* 1966). The highest heterologous reactions were to the *P. brasilianum* antigen and secondarily to *P. fieldi*. Such cross-reactions were also reported by Meuwissen (1968) using *P. falciparum*, *P. vivax*, *P. ovale*, *P. cynomolgi* (B strain), and *P. fieldi* antigens. In a further study, Sulzer *et al* (1969) found that a *P. malariae* antiserum gave a high level of reaction to *P. brasilianum*, followed by lower reactions to *P. vivax*, *P. falciparum*, and, finally, to *P. fieldi* antigens. Dranga *et al* (1969) in testing the cross-reactions between *P. vivax* and *P. malariae* antisera showed that a cross-reaction could always be observed between the 2 antigens but that the homologous responses were higher than the heterologous responses.

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(NS) = Not seen.