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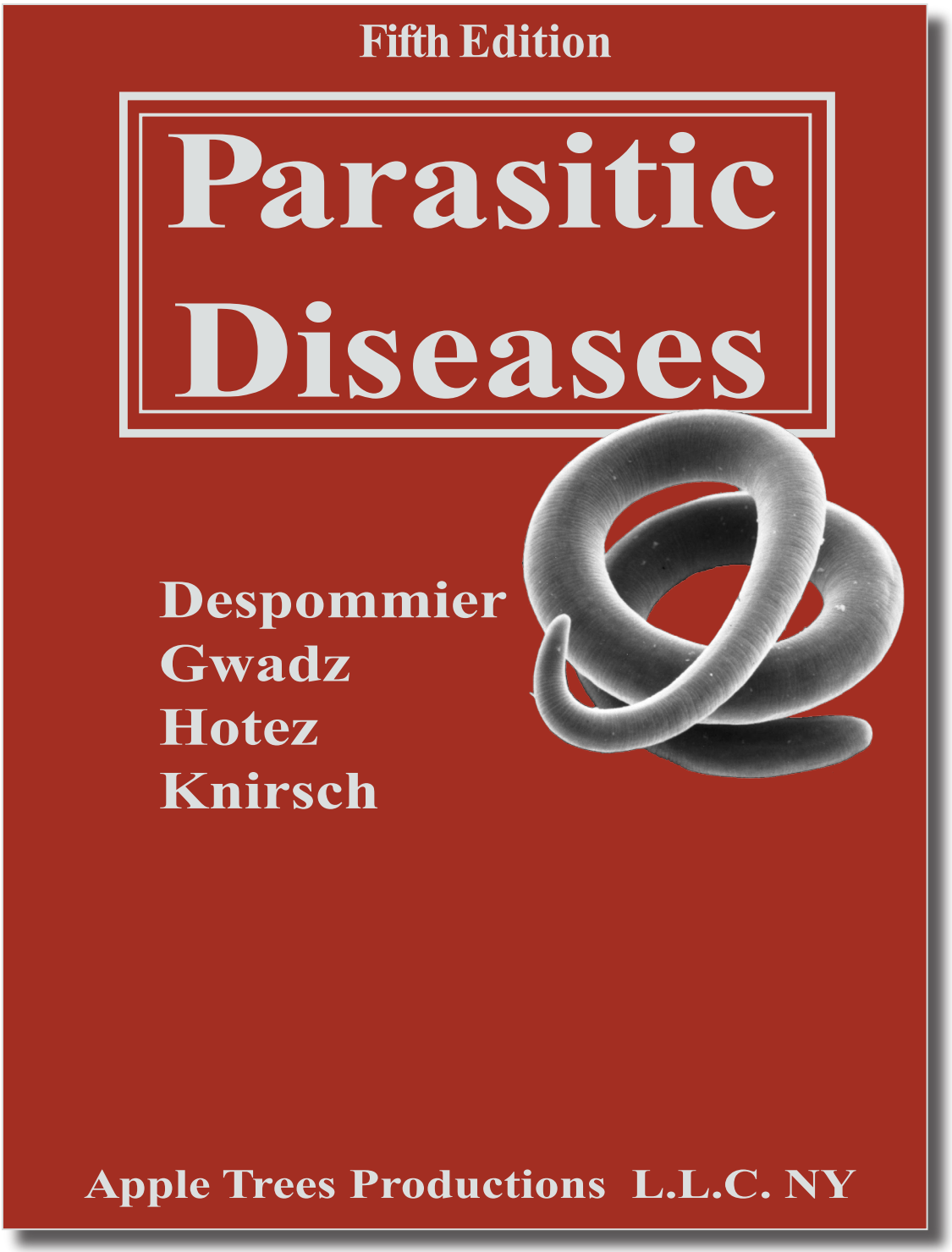


9. The Malarias:

Plasmodium falciparum
(Welch 1898)

Plasmodium vivax
(Grassi and Filetti 1889)

Plasmodium ovale
(Stephens 1922)

Plasmodium malariae
(Laveran 1881)

Introduction

Malaria is a mosquito-borne (Fig. 9.1) infection caused by protozoa of the genus Plasmodium. Humans are commonly infected by four species of the parasite*: P. falciparum, P. vivax, P. ovale*, and *P. malariae*. Certain species of Plasmodium that usually have simian hosts may also infect humans.

Malaria remains the most important parasitic infection and one of the most prevalent infectious diseases. More than 800 million cases and at least one million consequent deaths are estimated to occur annually, and more than one-half of the world's population lives in areas where malaria is endemic.[1, 2] Although formerly found throughout much of the world, with seasonal outbreaks extending well into temperate zones, malaria is now generally restricted to tropical and subtropical regions. However, travel and persistence of mosquito vectors in once-malarious areas continue to pose a threat of reintroduction of these parasites into non-immune populations.

Historical Information

Malaria most certainly afflicted man's ancestors. The earliest medical writers in China, Assyria, and India described malaria-like intermittent fevers, which they attributed to evil spirits. By the fifth century BC, Hippocrates was able to differentiate quotidian, tertian, and quartan fevers and the clinical symptoms of the disease.[3] At that time it was assumed that the disease was caused by vapors and mists arising from swamps and marshes. These theories persisted for more than 2,000 years and were reinforced by repeated observations that the draining of swamps led to a reduction in the number of cases of malaria. Indeed, the names for this disease, malaria (mal, bad; aria, air) and paludism (palus, marsh) reflect these beliefs.

Figure 9.1. Adult *Anopheles dirus* taking a blood meal from one of the authors (RWG).

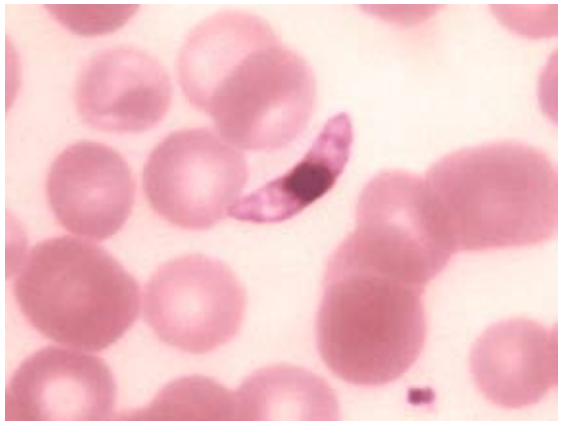


Figure 9.2. Gametocyte of *Plasmodium falciparum.*

All concepts of malaria changed within 20 years after Laveran's 1880 description of the crescent shaped sexual stage of *P. falciparum* and his observation of the dramatic release of the parasite's highly motile microgametes in the fresh blood of an infected soldier. Asexual development was described by Golgi in 1886, and the sexual cycle of the parasite was observed by MacCallum in 1897. In 1898 Ross, using a species of bird malaria, and Grassi and colleagues, working with human malaria, showed that the parasite developed in the mosquito and was transmitted by the bite of that insect. Ultimately, Ross and Laveran were awarded Nobel prizes for their contributions.[4, 5]

Most of the basic features of the life cycle of the malarial parasite were understood by 1900. The scientific efforts then shifted to attempts to control the disease. Early strategies mainly sought to reduce the number of mosquitoes. Another 30 years passed before the exoerythrocytic phase of the life cycle was described for a malaria of birds; it was 20 years later that the analogous stages were discovered in the simian and human livers.

Chemotherapy of malaria preceded the description of the parasite by nearly 300 years. The Peruvian bark of cinchona, or "fever tree", was first used during the early part of the seventeenth century, but the details of its discovery and its introduction into Europe are still a matter of discussion.[6-9] The alkaloids of the cinchona tree, quinine and cinchonine, were isolated in 1820 by Pelletier and Caventou. Synthetic antimalarial compounds effective against various stages of the parasite

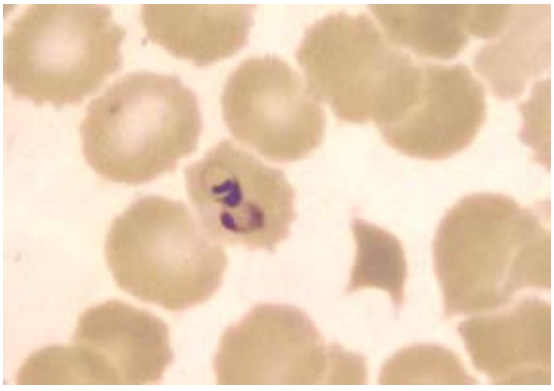


Figure 9.3. Signet ring stage of *Plasmodium spp.*

were later developed in Germany (pamaquine in 1924, mepacrine in 1930, chloroquine in 1934), in Britain (proguanil in 1944), and in the United States (pyrimethamine and primaquine in 1952).[9]

The Greeks and Romans practiced the earliest forms of malaria control, albeit inadvertently, by draining swamps and marshes. Their purpose was reclamation of land. These techniques were continued for centuries before the role of the mosquito as vector was discovered. Almost immediately, malarial control became synonymous with the control of mosquitoes. Destruction of breeding places by drainage and filling the swamps, killing the larvae by placing crude oil on the waters, and later by adding the larvicide Paris green, were typical early attempts. With the development of DDT, a residual insecticide, large-scale control programs became possible. They culminated in 1957 when the World Health Organization launched a worldwide eradication program.[12-15]

Plasmodium falciparum

Infection caused by *P. falciparum* (Fig. 9.2, 9.16) produces a form of malaria historically referred to as aestivoautumnal, malignant tertian, or simply falciparum malaria. It is the most pathogenic of the human malarias, and accounts for most of the mortality from the illness, and is the most prevalent of the human malarial infections. Falciparum malaria is now generally confined to tropical and subtropical regions and is the primary cause of malaria in sub-Saharan Africa.

Identification of *P. falciparum* is usually based on the presence of small ring-stage parasites on blood smears (Fig. 9.3). Infected erythrocytes are not enlarged, and multiple infections of a single erythrocyte are common. The rings often show two distinct chromatin dots. As trophozoites mature, they become sequestered in the capillaries of internal organs, such as the heart, brain, spleen, skeletal muscles, and placenta, where they complete their development. As a result of sequestration, maturing parasites usually are not present in the peripheral circulation. The appearance of the mature asexual stages (larger trophozoites and schizonts) in the peripheral circulation indicates the increasing severity of the disease.

Gametocytogenesis also proceeds in sequestered erythrocytes and requires approximately ten days. The falciparum gametocytes are characteristically crescentic, or banana-shaped (Fig. 9.2). They remain infectious for mosquitoes for as long as four days.

Falciparum malaria does not relapse; that is, the erythrocytes do not become reinfected from a persistent infection in the liver once the parasites are cleared from the blood by drugs or by the immune response of the host. However, recrudescences (reappearance of erythrocytes infected by the blood stages of the organism when maintained at low levels) are common and can recur for about two years.

Plasmodium vivax

Plasmodium vivax infection is called benign tertian or vivax malaria. Red blood cells infected with *P. vivax* (Fig. 9.4, 9.17) are enlarged and, when properly stained with Giemsa, often show stippling on the erythrocyte membrane, known as Schüffner's dots. All stages of the parasite are present in the peripheral circulation. Single infections of invaded erythrocytes are characteristic. Gametocytes appear simultaneously with the first asexual parasites. The duration of the viability of the sexual stages appears to be less than 12 hours. *Plasmodium vivax* produces the classic relapsing malaria, initiated from hypnozoites in the liver that have resumed development after a period of latency. Relapses can occur at periods ranging from every few weeks to a few months for up to five years after the initial infection. The specific periodicity of the relapses is a characteristic of the geographic strain of the parasite. Vivax malaria also has recrudescences due to persistent circulating erythrocytic parasites.

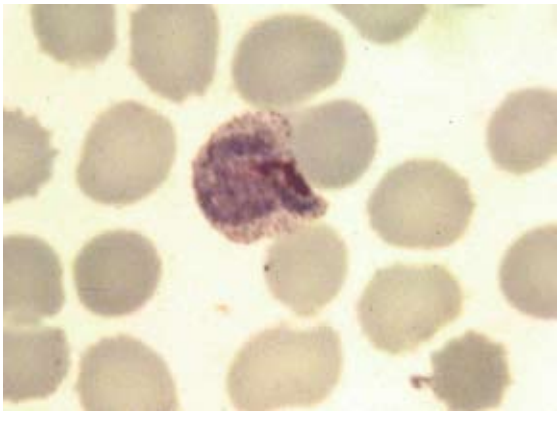
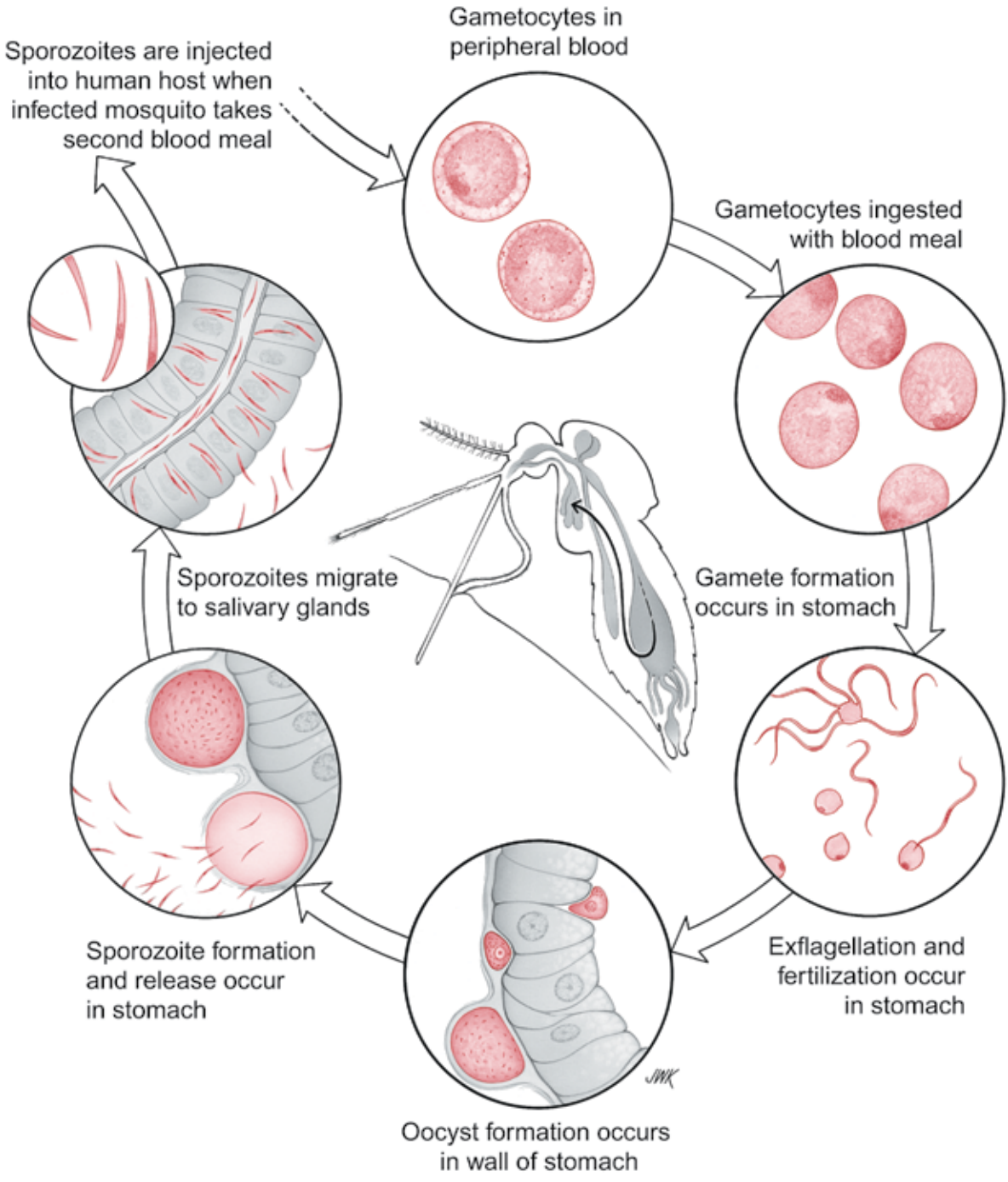


Figure 9.4. Trophozoite of *P. vivax*. Note Schüffner's dots in the parasite, and surrounding red cells that are smaller than the infected one.

Mosquito Cycle (Sporogany)



Plasmodium ovale

Plasmodium ovale (Fig. 9.5, 19.19) is the most recently described species of human malaria. Its distribution is limited to tropical Africa and to discrete areas of the Western Pacific. Ovale malaria produces a tertian fever clinically similar to that of vivax malaria but somewhat less severe. It exhibits relapses for the same duration as is seen with vivax malaria.

Plasmodium malariae

The disease caused by *P. malariae* is known as quartan malaria. *P. malariae* has a wide but spotty distribution throughout the world. Development in the mosquito is slow, and infection in humans is not as intense as those caused by the other Plasmodium species. Most current evidence indicates that *P. malariae* does not relapse. It does have recrudescences originating from chronic erythrocytic infections and can persist as a low level infection in the human host for decades.[10, 11] Erythrocytes infected with *P. malariae* remain the same size throughout schizozony (Fig. 9.6, 9.7, 9.18).

Simian Malarias That Infect Humans

Some species of Plasmodium that are parasites of chimpanzees and monkeys occasionally infect humans.[5] The disease they cause is relatively mild. Most notable is the quotidian fever (24-hour cycle) caused by *P. knowlesi* and the vivax-like malaria caused by *P. cynomolgi*.[12] Reports of human infection with malaria parasites from monkeys are becoming common with the ability to differentiate otherwise morphologically similar human and simian parasites at the molecular level. [13-15]

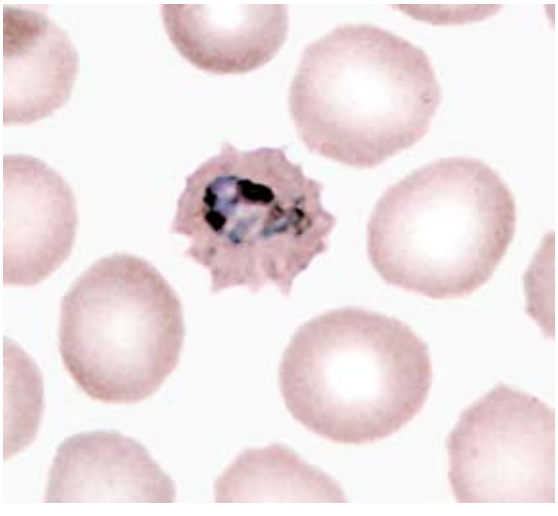


Figure 9.5. Trophozoite of *P. ovale*. Note "crenated" appearance of infected red cell. Courtesy M. Guelpe.

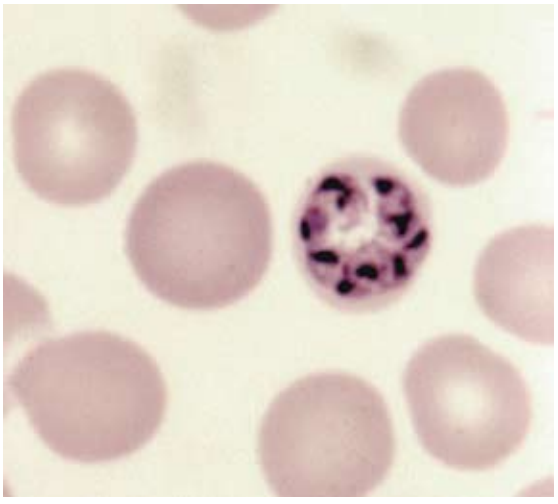


Figure 9.6. Schizont of *P. malariae*. Note red cells are the same size as the infected cell.

Life Cycles

The biology of the four species of Plasmodium is generally similar and consists of two discrete phases: sexual and asexual. The asexual stages develop in humans; first in the liver and then in the circulating erythrocytes. The sexual stages develop in the mosquito.

Asexual Stages

When the infected female Anopheles mosquito takes a blood meal (Fig. 9.1), she injects salivary fluids into the wound. These fluids contain sporozoites (Fig. 9.8), small (10-15 μm long), spindle-shaped, motile forms of the parasite, which initiate the infection. They are cleared from the circulation within an hour and eventually reach parenchymal cells of the liver. The route sporozoites follow to the liver has not been definitely established, and the hypotheses are subjects of controversy.[16-18] Once inside the liver cell, the parasites undergo asexual division (exoerythrocytic schizogony) (Fig. 9.9). The length of this exoerythrocytic phase and

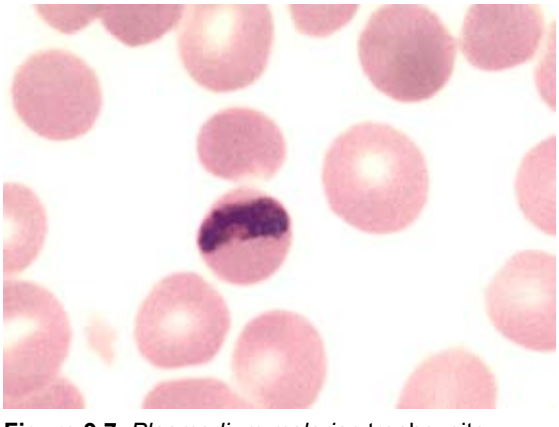
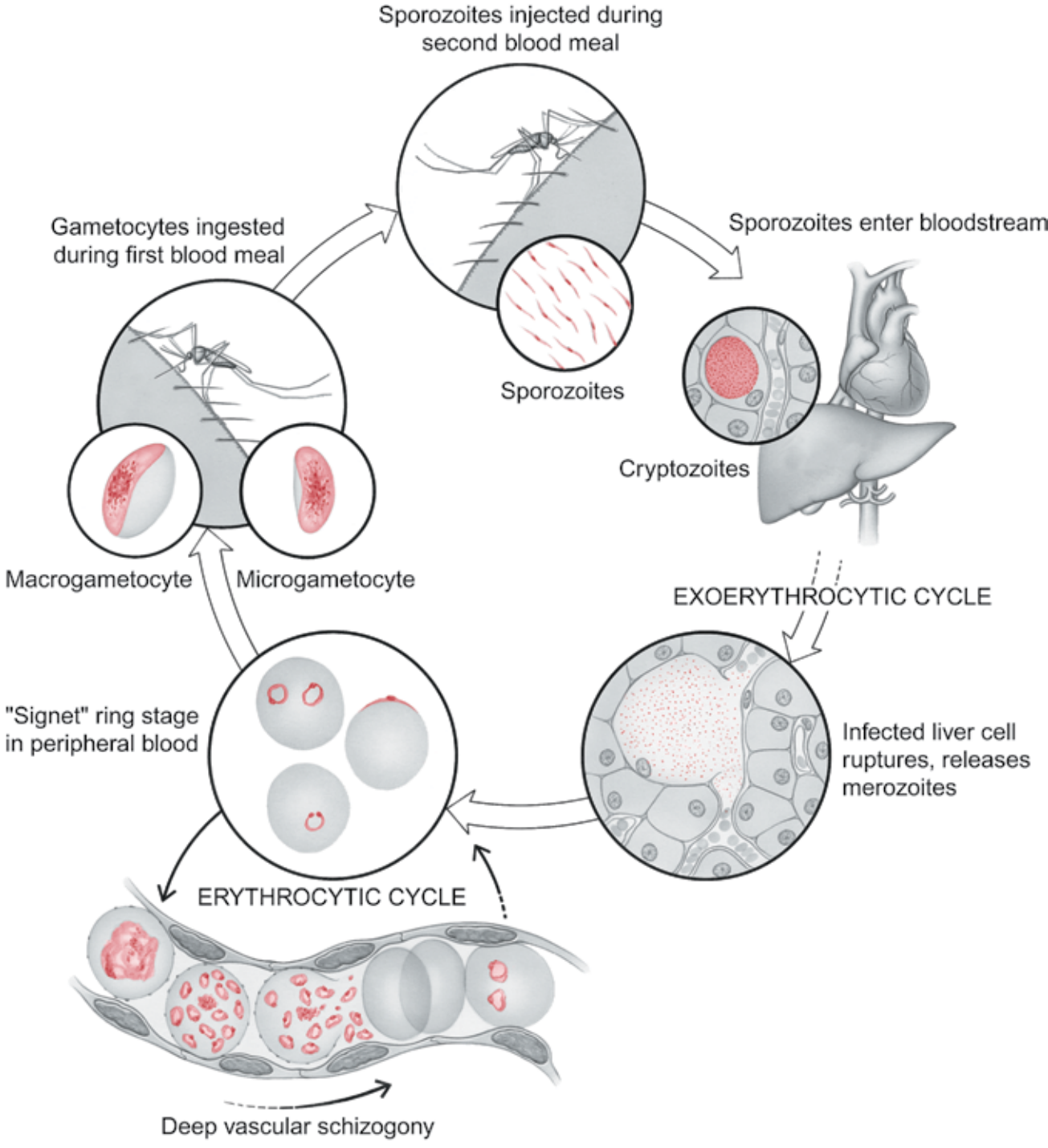


Figure 9.7. *Plasmodium malariae* trophozoite

Plasmodium falciparum



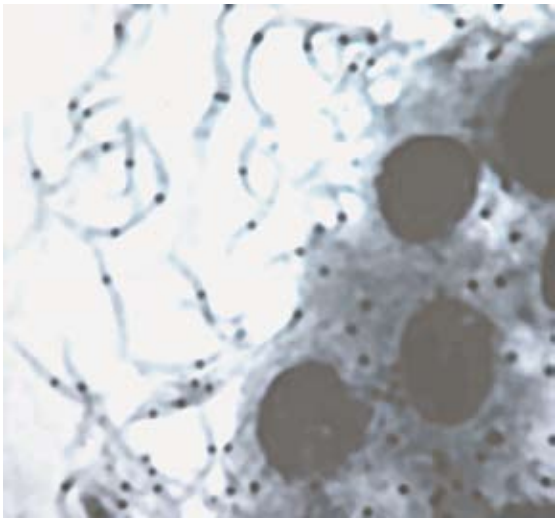


Figure 9.8. Sporozoites of malaria in infected mosquito stomach preparation.

the number of progeny (merozoites) produced within each infected cell is a characteristic of the individual species of Plasmodium. *P. vivax* can mature within 6-8 days, and each of its sporozoites produces about 10,000 daughter parasites. For *P. ovale*, these values are 9 days and 15,000 merozoites; for *P. malariae*, 12-16 days and 2000 merozoites; and for *P. falciparum*, 5-7 days and 40,000 merozoites.

The phenomenon of relapse in certain malarias (*P. vivax, P. ovale, P. cynomolgi*) has not been fully explained. By definition, a parasitologic malarial relapse is the reappearance of parasitemia in sporozoite-induced infection, following adequate blood schizonticidal therapy.[19] It has been long accepted that the exoerythrocytic forms of relapsing malaria persist in the liver as a result of cyclic development (rupture of infected cells and invasion of new cells).[20] However, experimental evidence has lent support to a different hypothesis for the mechanisms of relapse. It holds that some sporozoites fail to initiate immediate exoerythrocytic development in the liver and remain latent as the so-called hypnozoites capable of delayed development and initiation of relapse.[21] Several patterns of relapse have been described, often related to the geographic origin of the parasite; temperate strains of *P. vivax* may show delayed primary attacks and relapses, whereas more tropical forms emerge from the liver within weeks of infection. In vivax and ovale malarias, eradication of parasites from the peripheral circulation with drugs aborts the acute infection. Subsequently a fresh wave of exoerythrocytic merozoites from the liver can reinitiate the infection. The dormant parasites, or hypnozoites can remain quiescent in the liver for as long as five years. To achieve radical cure, it is necessary to destroy not only the circulating parasites but also the hypnozoites.

Plasmodium falciparum and *P. malariae* do not develop hypnozoites, and therefore lack the capacity to relapse. Untreated *P. falciparum* can recrudesce for 1-2 years through the continuation of the erythrocytic cycle, which for periods of time remains at a subclinical, asymptomatic level; *P. malariae* can do so for 30 years or more.[10] For both infections radical cure can be achieved by drugs that need only to eradicate the parasites in the peripheral circulation.

Erythrocytic Phase

When merozoites are released from the liver schizonts, they invade red blood cells (Fig. 9.10) and initiate the erythrocytic phase of infection. Invasion of the erythrocytes consists of a complex sequence of events beginning with contact between a free-floating merozoite and the red blood cell.[22] Attachment of the merozoite to the erythrocyte membrane involves interaction with specific receptor sites. Thereafter the erythrocyte undergoes rapid and marked deformation. The parasite enters by a localized endocytic invagination of the red blood cell membrane, utilizing a moving junction between the parasite and the host cell membrane.[23]

Once within the cell, the parasite begins to grow, first forming the ring-like early trophozoite, and eventually enlarging to fill the cell. The organism then undergoes asexual division and becomes a schizont composed of merozoites. The parasites are nourished by the hemoglobin within the erythrocytes and produce a characteristic pigment called hemazoin. The erythrocytic cycle is completed when the red blood cell ruptures and releases merozoites that proceed to invade other erythrocytes.[24]

The asexual cycle is characteristically synchronous and periodic. *Plasmodium falciparum, P. vivax*, and *P. ovale* complete the development from invasion by merozoites to rupture of the erythrocyte within 48 hours, exhibiting “tertian” periodicity. *Plasmodium malariae*, which produces “quartan” malaria, requires 72 hours for completion of the cycle.

Infection with erythrocytic phase merozoites can

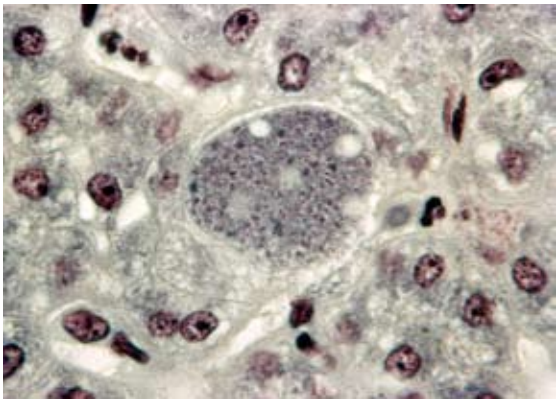
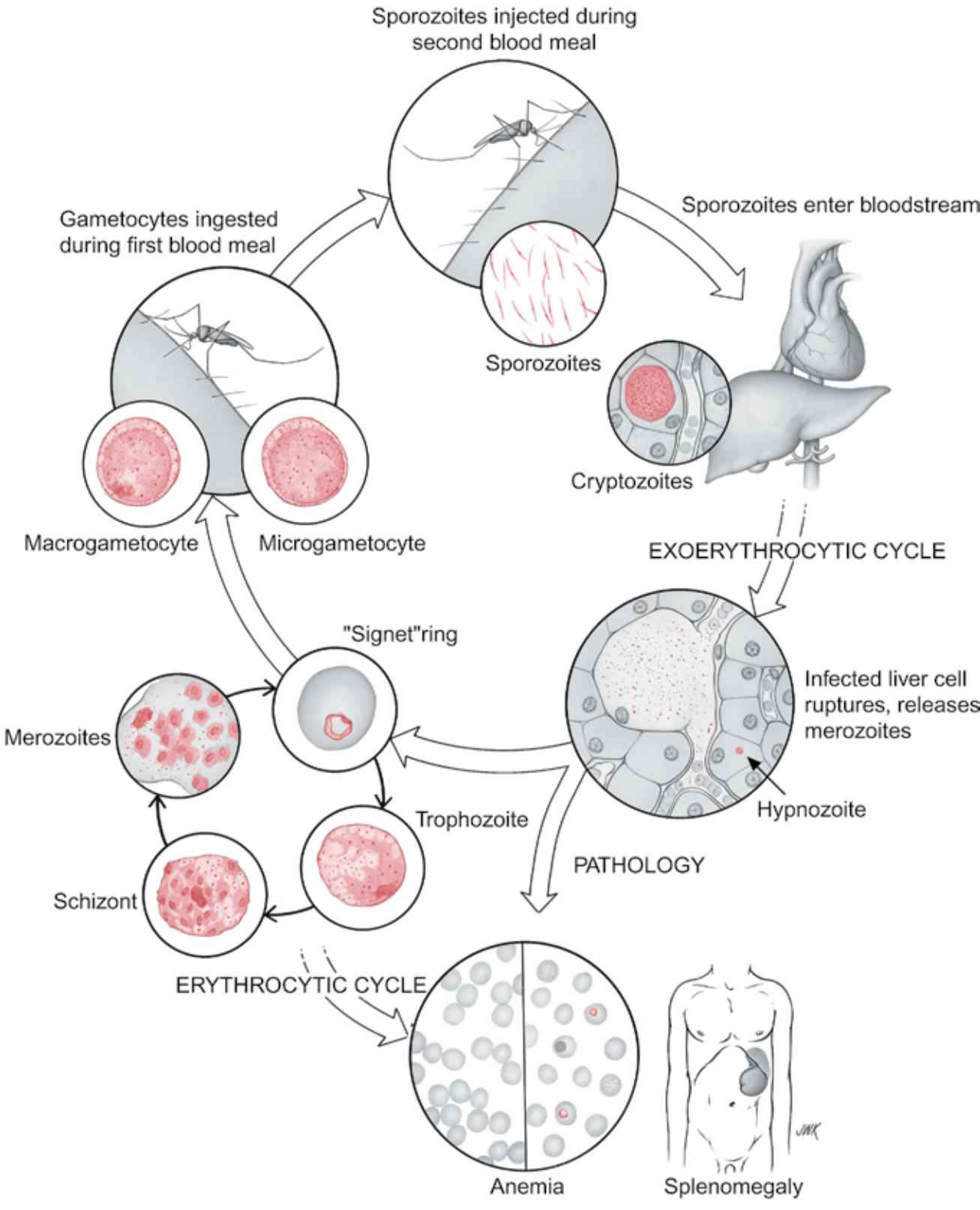


Figure 9.9. Exoerythrocytic stages of malaria in liver parenchymal cell.

Plasmodium vivax



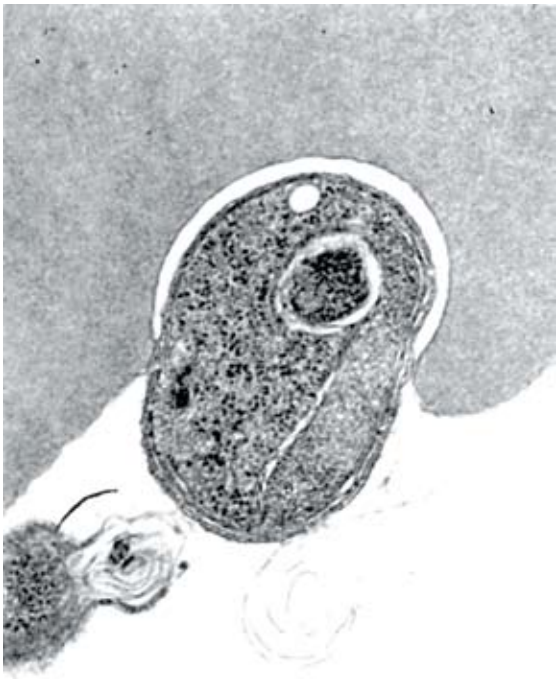


Figure 9.10. Transmission EM of a merozoite entering a red cell. Note points of attachment. Courtesy S. Langreth.

also occur as a result of blood transfusion from an infected donor or via a contaminated needle shared among drug addicts. Malaria acquired in this manner is referred to as "induced" malaria.[25] Congenital malaria as a result of transplacental infection rarely occurs.[26]

Sexual Stages

Not all merozoites develop asexually. Some differentiate into the sexual forms – macrogametocytes (female) and microgametocytes (males) – which can complete their development only within the gut of an appropriate mosquito vector. On ingestion by the mosquito in the blood meal, the gametocytes shed their protective erythrocyte membrane in the gut of the vector. Male gametocytes initiate exflagellation (Fig. 9.11), a rapid process that produces up to eight active, sperm-like microgametes, each of which can eventually fertilize the macrogametes. The resulting zygotes elongate into diploid vermiform ookinetes, which penetrate the gut wall and come to lie under the basement membrane (Fig. 9.12). The parasites then transform into oocysts within 24 hours of ingestion of the blood meal. Development of sporozoites follows, leading to the production of more than 1,000 of these now-haploid forms in each oocyst. They mature within 10–14 days, escape from the oocyst, and invade the salivary glands. When the mosquito bites another human host, a new cycle begins.

Although the four species have marked physiologic differences and some major differences in the pathologic course they pursue, they are most simply differentiated on the basis of their morphology. Thus, the blood smear, typically fixed and stained with Giemsa or Wright solution, is the basis of the fundamental diagnostic test, although alternatives are available. Commercially available methods for malaria parasite detection and characterization are becoming increasingly sensitive, and may eventually supplant microscopy in more advanced laboratories (see Diagnosis).[27]

Cellular and Molecular Pathogenesis

The rupture of infected erythrocytes and release of pyrogens are accompanied by fever and the consequent chills and sweating associated with malaria. The pathogenesis of general malaise, myalgia, and headache is not clear. The characteristic periodicity of the fever, based on synchronous infections, is not invariable; the early phases of all infections are often not synchronous. Some infections may be due to two or more broods of parasites, with the periodicity of one independent of that of the others. With severe falciparum malaria, the patient may be febrile continuously

Cerebral malaria is the most consequential manifestation of severe falciparum infection.[29] It is caused by blockage of the cerebral capillaries with infected erythrocytes, which adhere to the endothelium.[30] The mechanism of cytoadherence is related to the presence of "knobs" on the surface of the infected red blood cells and their subsequent attachment to appropriate receptors on the host endothelium (Fig. 9.13, 9.14). Although not essential for cytoadherence, the knobs seem to enhance binding.[31] They are produced by the parasite and host and consist of part of a histidine-rich protein.[32, 33] Binding to endothelial cells involves several host cell receptors, including CD36, thrombospondin,

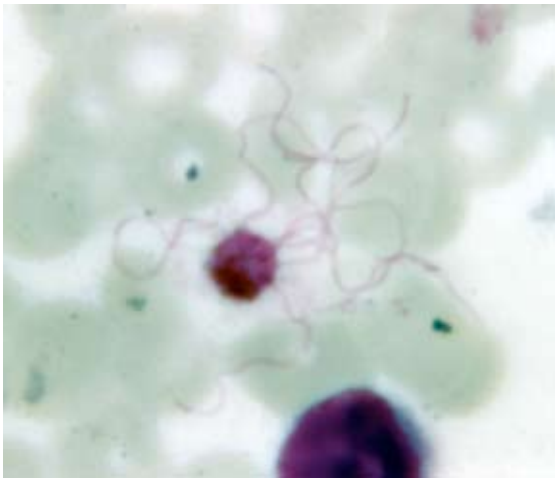


Figure 9.11. Exflagellation of the microgametocyte of a malaria parasite. Each "flagella" is actually a male gamete.

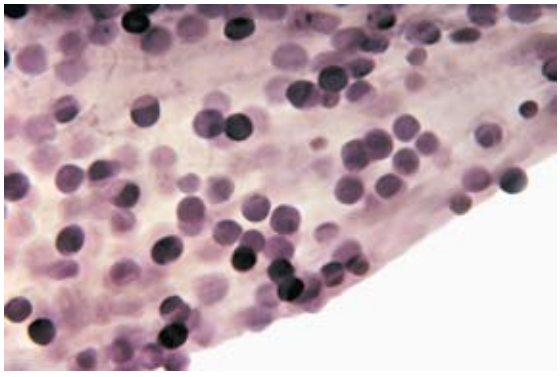


Figure 9.12. Portion of an infected mosquito stomach. Note numerous oocysts on outer wall.

intercellular adhesion molecule 1 (ICAM1), and others. Cytoadherance in the placentae of women in their first preganacies involves parasite binding to chondroitin sulfate A (CSA) and does not appear to involve the erythrocyte knobs.[34, 35] Sequestration of malaria parasites in the placentae of primagravid females is a major cause of death, fetal mortality, fetal wastage and low birth weight.[35-37] With falciparum malaria, anemia caused by hemolysis can be severe. Damage to the erythrocytes by intravascular hemolysis often is greater than that caused by rupture of the infected cells alone. Even uninfected cells have an increased osmotic fragility. Also present is bone marrow depression, which contributes to the anemia. Disseminated intravascular coagulopathy occurs in severely infected individuals.

The spleen plays a major role in host defense against malaria (Fig. 9.15). Parasitized cells accumulate in its capillaries and sinusoids, causing general congestion. Malarial pigment becomes concentrated in the spleen and is responsible for the darkening of this organ. Chronic infection, particularly with *P. malariae*, often causes persistent splenomegaly and is responsible for "big spleen disease," or tropical splenomegaly

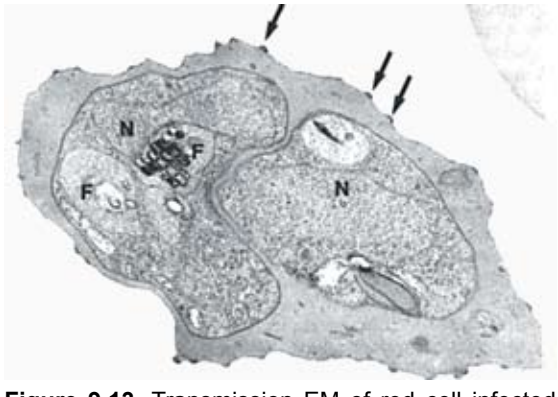


Figure 9.13. Transmission EM of red cell infected with *P. falciparum*. Arrows indicate points of attachment to host endothelial cells. N=nucleus, F=food vacuole. Courtesy S. Langreth.

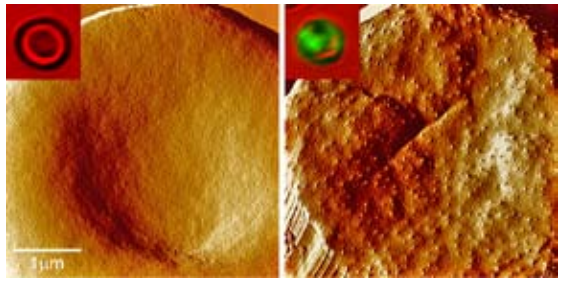


Figure 9.14. Atomic force microscopy of normal (left) and *Plasmodium falciparum*-infected (right) red cells. Courtesy J. Dvorak.

syndrome,[38] consisting of hepatomegaly, portal hypertension, anemia, leukopenia, and thrombocytopenia. With vivax malaria, the spleen can become acutely enlarged and is susceptible to rupture. The liver is darkened by the accumulated malarial pigment and shows degeneration and necrosis of the centrilobular regions. The gastrointestinal tract is also affected. There are focal hemorrhages, edema, and consequent malabsorption. The kidneys, particularly with severe falciparum malaria, show punctate hemorrhages and even tubular necrosis. Accumulation of hemoglobin in the tubules is responsible for hemoglobinuria, or blackwater fever[39], which occurs after repeated attacks of falciparum malaria and is complicated by therapy with quinine. It is a consequence of severe hemolysis exacerbated by the host immune response against the intracellular parasites.

Chronic infections with *P. malariae* can lead to nephrotic syndrome, characterized by focal hyalinization of the tufts of glomeruli and by endothelial proliferation, apparently caused by the deposition of immune complexes. In addition, evidence suggests that an autoimmune process develops against glomerular basement membrane.

Congenital malaria can develop with any of the species of Plasmodium, although the incidence of this complication is relatively low. The mechanism by which the fetus becomes infected is uncertain. Some investigators have postulated damage to the placenta as a prerequisite to congenital malaria, but it is also possible that the parasites can infect the fetus through an intact placenta or at the time of birth. Malarial infections tend to suppress cell-mediated immune responses. It has been suggested that Burkitt's lymphoma is caused by infection with the Epstein-Barr virus under the influence of immunosuppression by chronic falciparum malaria.[40]

HIV and Malaria

The relationship between infection with malaria and the human immunodeficiency virus is a subject of great interest and intense scrutiny. In Africa, these two agents overlap in their geographic ranges and the populations

they infect. Both are significant infections of children and young mothers. Indeed, it is fair to assume that most individuals infected with HIV were already infected with malaria. There is new evidence to suggest a relationship between HIV-induced reduction of CD4 cell counts and a rise in incidence of malaria.[41-45]

Clinical Disease

The most pronounced clinical manifestations of adult-onset malaria are periodic chills and fever, usually accompanied by frontal headache and myalgia.[27] Fever may persist for several days before the typical periodicity develops. In contrast, young children often present with non-specific symptoms, including fever, cough, vomiting, and diarrhea. Symptoms of malaria usually first appear 10-15 days after the bite of the infected mosquito, although delays of several months in the onset of symptoms and the appearance of parasites in peripheral blood are common, particularly for some strains of *P. vivax* found in temperate zones. Patients undergoing chemoprophylaxis may not develop any symptoms until they stop taking the drug. The classic pattern of clinical disease consists of paroxysms of chills and fever, reaching 41°C and lasting six hours, followed by sweating and defervescence. There are exceptions to this pattern, however, as noted under the pathogenesis section.

Additional symptoms for all malarias include malaise, nausea, anorexia, and abdominal pain. Vomiting can also develop and may be intense. Initially, there can be mild anemia with an elevation of the reticulocyte count. The leukocyte count tends to be normal or even low; there is no eosinophilia.

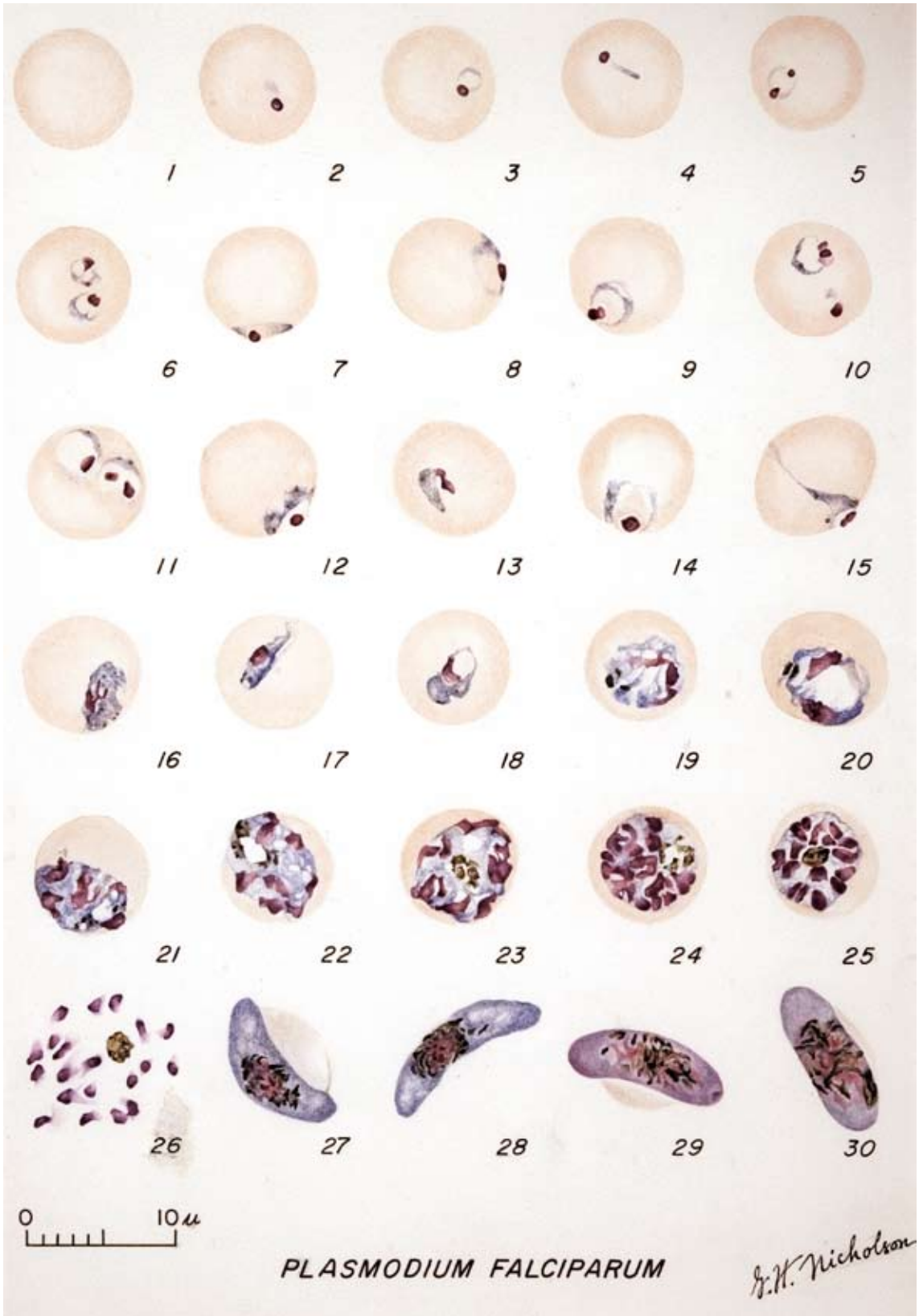
Figure 9.15. Child infected with malaria, probably *P. malariae*. Note enlarged spleen.

All forms of untreated malaria tend to become chronic. Repeated attacks are caused by recrudescence or relapses. The development of immunity eventually leads to spontaneous cure of falciparum malaria within two years and of vivax and ovale malarias within five years, although individuals are susceptible to reinfection during and after this period (Fig. 9.20). Infection with the quartan parasite can persist 30 years or more. Untreated falciparum malaria can be fatal during the initial attack, an especially likely event in young children (Fig. 9.20).

Unexplained fever in patients who have received transfusions or who are drug addicts may signal the presence of induced malaria. An infant who develops fever during the neonatal period should be suspected of malaria if the mother had been exposed to this infection. Diagnostic tests for induced or congenital malaria are the same as for the conventional forms of the disease. It must be remembered that neither induced nor congenital malaria has an exoerythrocytic cycle in the liver; hence, therapy directed against the liver cycle is inappropriate.Innate resistance to malaria is mediated by factors other than immune mechanisms. *Plasmodium vivax* and *P. ovale* preferentially invade reticulocytes. With these infections, usually only about 2% of red blood cells are parasitized, and the clinical disease is relatively mild. In contrast, *P. malariae* tends to invade older erythrocytes, again limiting maximum parasitemia. Finally, *P. falciparum* attacks erythrocytes of all ages, permitting high levels of parasitemia.

There are a number of genetic factors in human populations which confer varying levels of resistance to malaria.[46-48] Individuals carrying the gene for sickle-cell hemoglobin receive some protection against falciparum malaria. Those with sickle-cell trait (A and S hemoglobins) have a selective advantage over those with the hemoglobin AA genotype because the heterozygotes are protected against the severity of malaria. The hemoglobin SS individuals are also protected, but their sickle-cell disease leads to early death.[46-48] In areas of Africa with the highest frequency of this gene, it is estimated that the death rate due to malaria needed to have fixed this gene frequency may have exceeded 25%.[49] This situation is an example of balanced polymorphism.

The precise mechanism for the protection afforded by sickle hemoglobin S remains obscure, although it appears that both physiologic and immunologic factors may play a role. At the same time, hemoglobin AS individuals with *P. falciparum* infection may have lower cellular activation and higher cellular reactivity in response to malarial antigens.[50, 51]Hemoglobin C mutations appear to provide similar protection against falciparum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G.H. Nicholson

Figure 9.16.

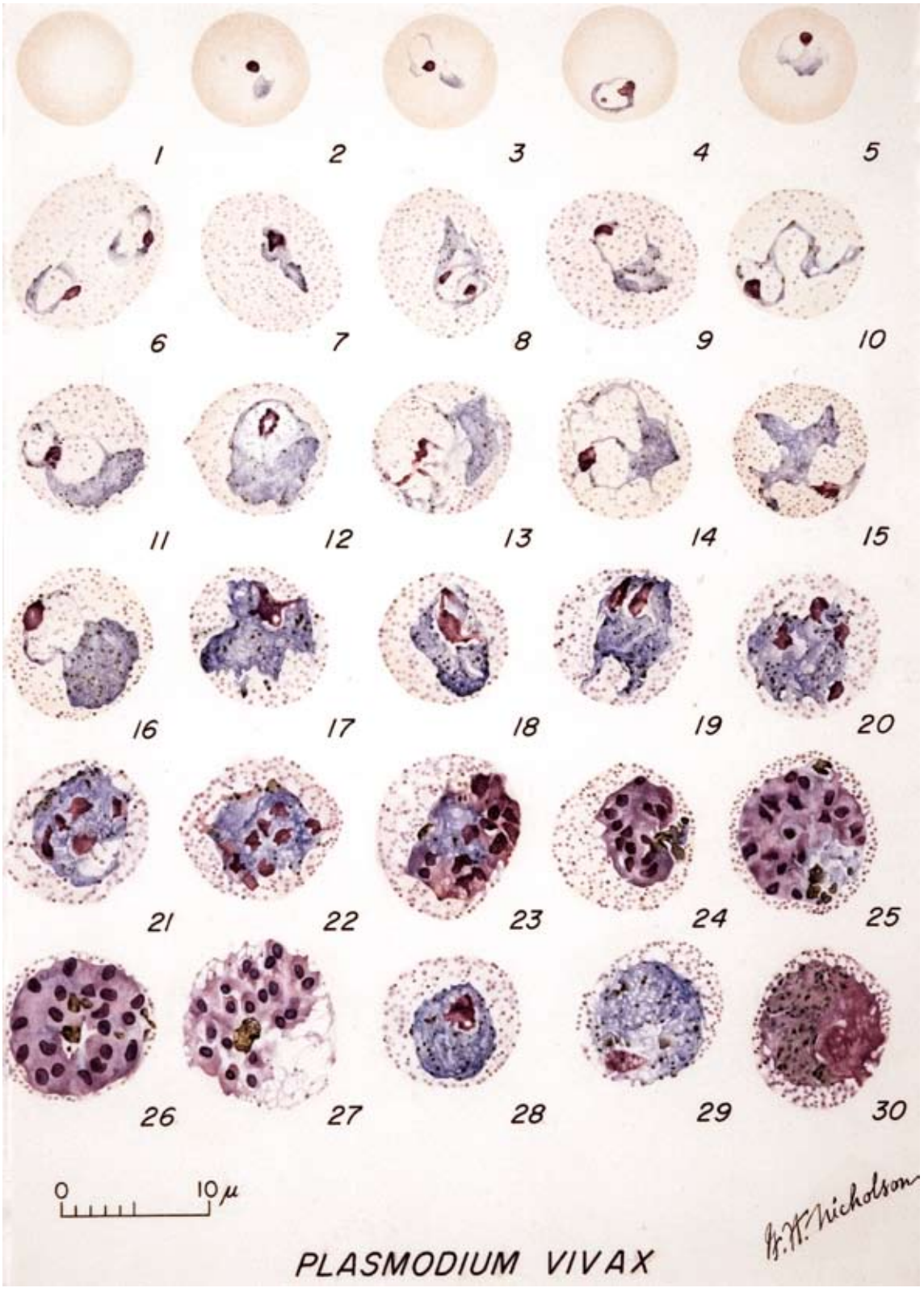
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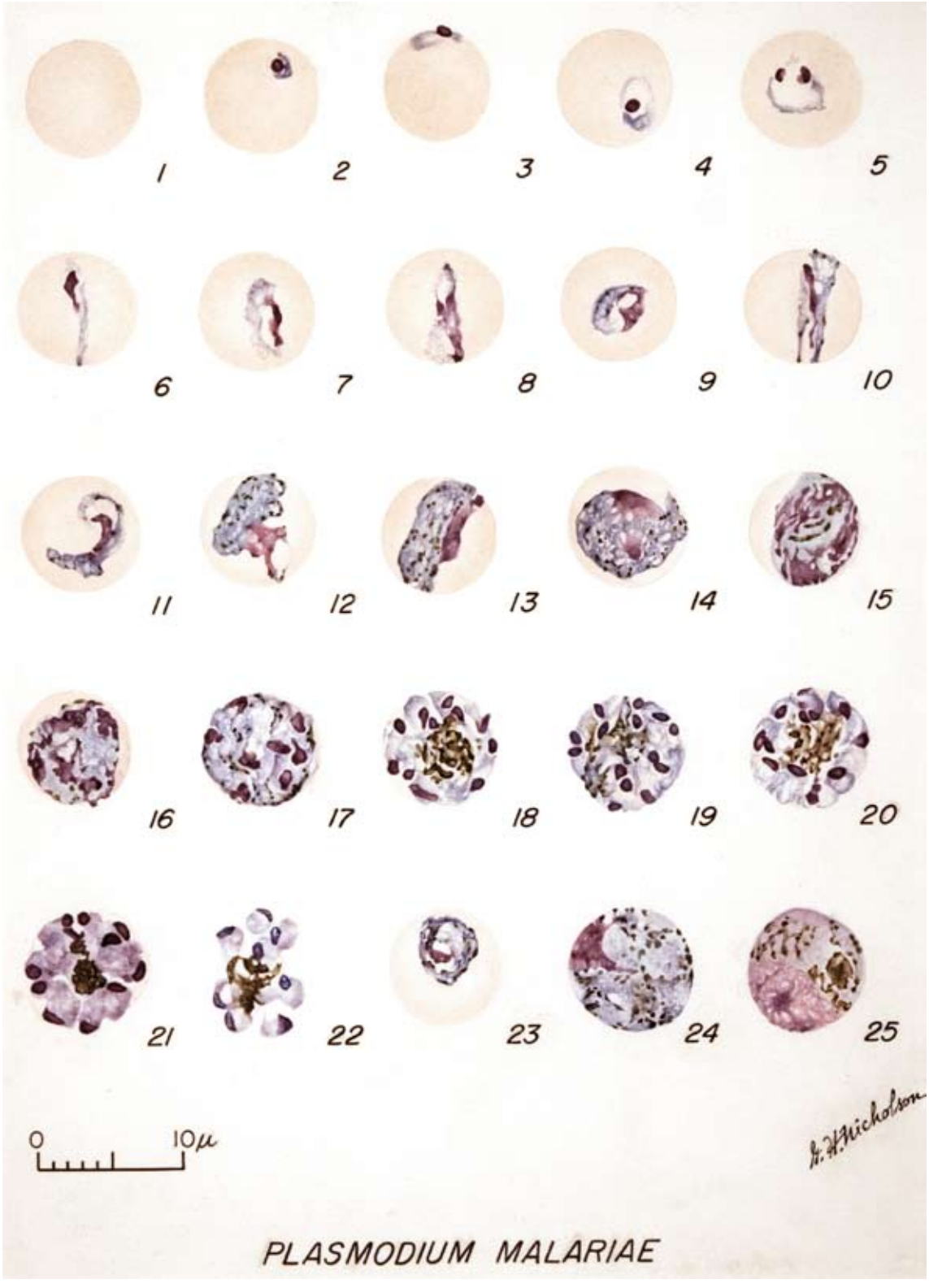
Figure 9.17.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G. H. Nicholson
PLASMODIUM MALARIAE

Figure 9.18.

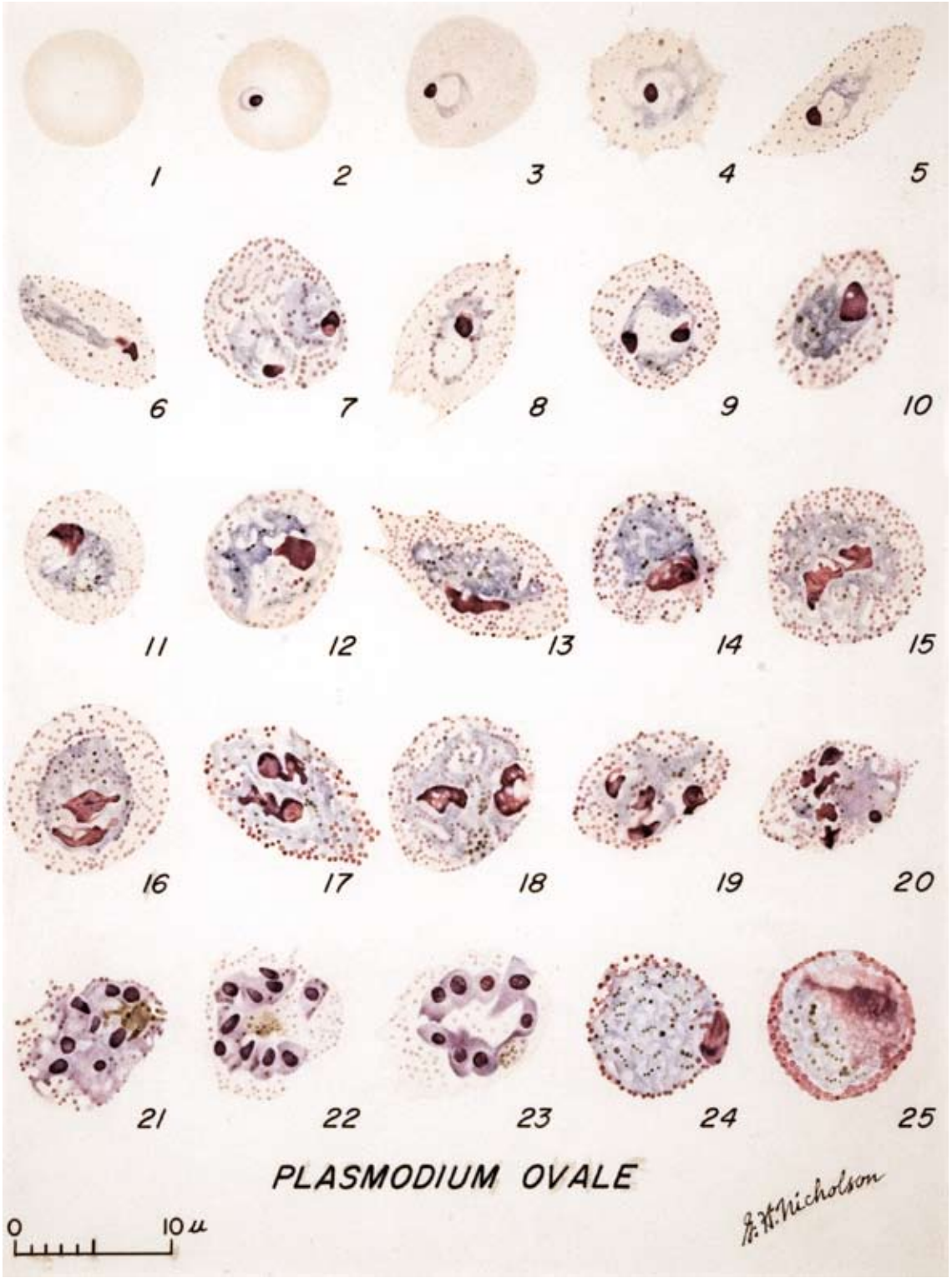
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Figure 9.19.

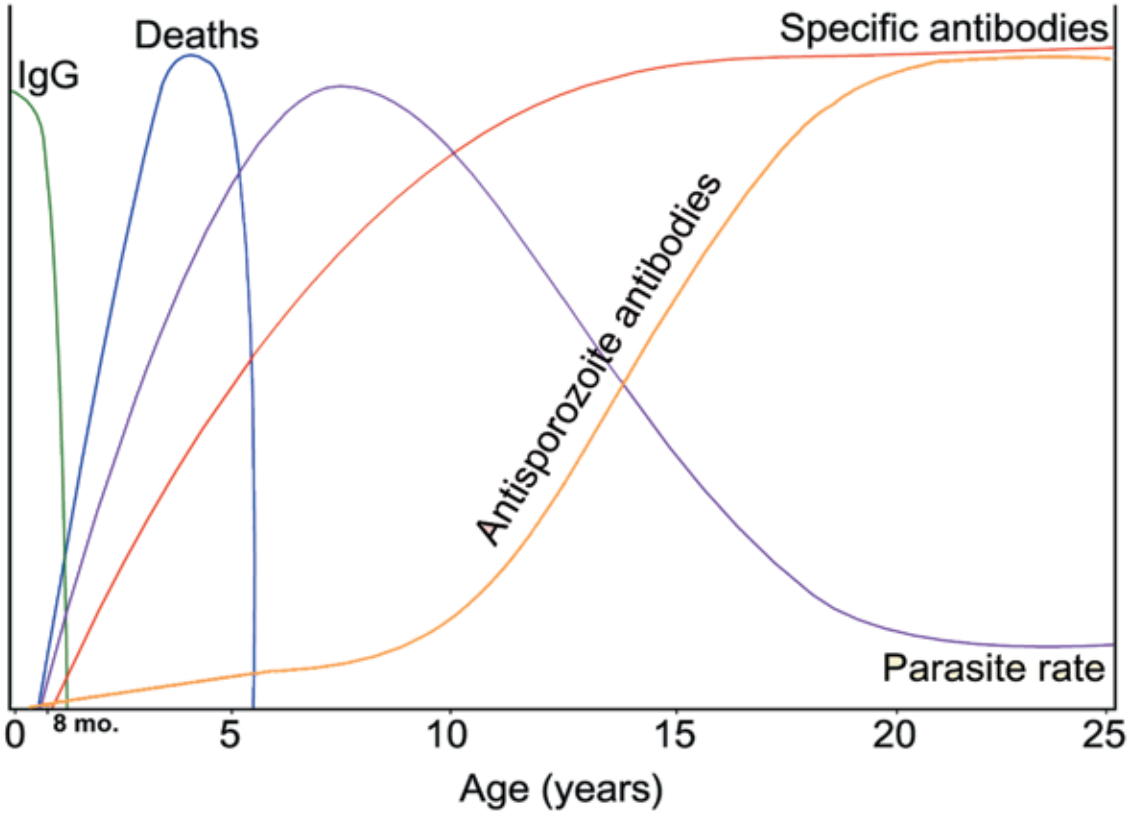

Figure 9.20. Graph indicating relationships between age of patient, susceptibility to infection, production of antibodies against different stages of parasite, and lethality of infection.

malaria in certain ethnic groups. [52,53]

Glucose-6-phosphate dehydrogenase deficiency, β-thalassemia, and ovalocytosis, the latter common in Southeast Asia,[54] have been implicated as mediators of innate resistance against *P. falciparum* infection. It has been suggested that the protective effect of thalassemia may be related to enhanced immune recognition and clearance of parasitized erythrocytes.[55]

Duffy blood type determinants are associated with receptor sites for *P. vivax* merozoites on the erythrocytes. While it is doubtful that the blood group carbohydrate itself is the actual receptor, most West Africans are negative for the Duffy blood type and are not susceptible to infection with *P. vivax*.[56]

Acquired immunity to malaria develops after long exposure and is characterized by low levels of parasitemia. Immune individuals have intermittent parasitemia with only mild symptoms. This state has been referred to as premunition, in contrast with classic immunity, which prevents any degree of infection.

Diagnosis

For over a century a definitive diagnosis of malaria has depended on the microscopic identification of the parasite on Giemsa-stained blood smears (see Appendices B and C). This procedure permits not only the confirmation of the presence of the parasite, but makes possible the identification of the species of malaria and an indication of the level of parasitemia in the infected host. Under normal conditions, both thick and thin smears should be examined. When malaria is suspected, the clinician should take a thorough travel history. Should the initial blood smears prove to be negative, new specimens should be examined at 6-hour intervals.

Identification of malaria on thick and thin blood smears requires an experienced microscopist who is well trained in parasite morphology. A British study indicated that at least 10% of positive slides are not identified.[57] To facilitate diagnosis, a number of techniques have been introduced, particularly for use in clinical laboratories. Microscopy enhanced with fluorescent stains, dipstick antigen detection, PCR assays, and the use of specialized blood cell analyzers are available and are constantly being improved. All can increase the probability of correct diagnosis, but still require microscopic confirmation.

In endemic areas, examination of stained blood films remains the standard. The application of more

sophisticated detection methods to endemic areas may be premature. Most of the newer methods require specialized reagents or equipment and are prohibitively expensive. At the same time, in endemic areas, even microscopic techniques are often not used; most fevers as assumed to be malarial and are recorded as such in clinic or hospital records.

The identification of parasite genes conferring resistance to antimalarial drugs has permitted the development of mutation-specific PCR primers that can readily identify resistant parasites. Such detection systems are available and in use in the field for pyrimethamine, sulfadoxine, cycloguanil and chloroquine.[58-60] Epidemiologic studies requiring information on sporozoite inoculation rates by mosquito vectors have been facilitated by ELISA systems using species-specific monoclonal antibodies directed against the predominant surface antigens of the sporozoites.[61] The capacity for rapidly determining the proportion of mosquito populations coming to feed that have infectious sporozoites in their salivary glands allows accurate prediction of risk of infection or assessment of the effects of intervention strategies in the control of malaria.

Treatment

Recommendations for malarial prophylaxis and treatment are in a constant state of flux. The long reliance on chloroquine to treat *P. falciparum* is no longer tenable, due to the worldwide spread of resistance.[62] Alternative drugs may be indicated on a region-by-region basis.[63] Fansidar, a pyrimethamine-sulfadoxine combination, has already lost effectiveness as a replacement for chloroquine in East African countries.[64] Malarone (atovaquone-proguanil) is now a recommended prophylactic drug for travelers to most areas where *P. falciparum* is resistant to chloroquine; side effects and resistance have been reported.

In cases of infection with chloroquine-resistant *P. falciparum* or with a parasite whose resistance cannot be determined, the treatment consists of quinine sulfate and a second agent that often includes pyrimethamine-sulfadoxine, tetracycline or clindamycin. Strains resistant to all of these drugs have been reported.[65]

Artemisinin derivates are now recommended as primary therapy in areas of high drug resistance and must be used in combination with a second agent to prevent recrudescence. [66, 67] If a patient has *P. falciparum* infection and is from anywhere in Central America, chloroquine can be used, as there is little resistance to the drug there. In the rest of the world, resistance to chloroquine is so frequently reported that every patient must be assumed to have this form of *P. falciparum*. If the origin of infection is unknown, as may be the case with induced malaria, one must treat the infection as if the organisms were resistant. For patients who are too ill to take drugs orally, chloroquine hydrochloride must be administered intramuscularly. For resistant falciparum malaria, intravenous quinidine or quinine dihydrochloride must be used. Intravenous administration of quinine is dangerous because the drug can cause fatal arrhythmias. It is mandatory to administer it slowly over a period of one hour and to monitor the patient's vital signs. Intravenous preparations of artemisinins have been studied and in pilot studies appear safe and effective although use in routine clinical practice will depend on larger controlled studies and a reliable production source.[68]

Relapses due to *P. vivax* infections can be prevented by a course of primaquine to eradicate the hypnozoite liver stage of this species. It need not be started immediately but can be deferred until the patient has recovered from the acute attack. Primaquine tends to cause hemolysis in individuals deficient in glucose-6-phosphate dehydrogenase; therefore, the drug should not be administered until the proper test for this enzyme has been performed. Patients who are deficient should be given an option of chloroquine prophylaxis for five years or treatment of each acute attack during the five years within which the infection is expected to relapse.

Prevention and Control

There are more than 300 species of Anopheles mosquitoes, but only about 60 species are considered important vectors of malaria. Some of the factors that influence the efficiency of the insect are their feeding habits (most importantly, a preference for human blood), longevity, and susceptibility to infection with the malarial parasite, and the size of the mosquito population.

The variability of the parasite plays an important role in the pathogenicity of the disease. For example, geographic strains of *P. vivax* show markedly different incubation periods and patterns of relapses, and *P. falciparum* shows considerable variability in its responses to anti-malarial drugs. The susceptibility of geographic strains of vector mosquitoes may be highly variable.

Malaria in the United States since the 1960s has been of the imported variety. The wars in Korea and Vietnam increased the numbers of these imported cases because of the returning service personnel. Refugees or immigrants from endemic areas constitute the largest number of imported cases. In addition, there is a steady incidence of malaria among travelers returning from endemic areas. Autochthonous infections are rare in the United States, despite large, persistent populations of the anopheline vectors, *Ann. quadrimaculatus* in the East and *An. freeborni* in the West. Outbreaks of *P. vivax* in southern California have been associated with a new vector species, *An. hermsi*.[69] There are regular reports of malaria transmission in the U.S.,

usually limited to one or two cases transmitted by local anopheline mosquitoes.

Travelers to endemic areas and short-term residents of such areas should follow the Centers for Disease Control and Prevention (CDC) guidelines. Prophylactic drugs must be taken prior to departure, during the stay in the endemic area, and for several weeks thereafter, the regimen being dictated by the choice of drug. As a primary precaution, travelers should avoid or minimize contact with mosquitoes. Since most anophelines bite at night, sleeping under netting and, if possible, in rooms fitted with window screens is effective. Clothing that covers much of skin and insect repellents, particularly those containing diethyltoluamide (DEET), are useful adjuncts to transmission prevention.

Differential diagnosis of a febrile illness in travelers to endemic zones after they have returned home should include malaria. Delays in diagnosis, particularly of falciparum malaria, have resulted in death. Similarly, fevers in individuals who have received blood transfusions or who are drug addicts must also be evaluated for the possibility of malaria.

The development of *P. falciparum* resistance to chloroquine has had serious effects on the general prevention and treatment of this disease. Multiple drug resistance is common in Southeast Asia, and is rapidly spreading in the Asian subcontinent, South America, and Africa. Reports of chloroquine, primaquine, and pyrimethamine resistance in *P. vivax* in South Asia raises the possibility that these drugs may eventually be lost as weapons in the fight against this important parasite.[70]

Controlling the mosquito vector remains the most practical method for wide-scale control of malaria. A reduction in the number of mosquitoes through drainage or modification of breeding sites has been accomplished in some areas. Insecticides still offer the best but increasingly less-acceptable method for reducing populations of mosquitoes or of interrupting transmission by targeting only those infected mosquitoes coming to feed in houses. However, the rising costs of these insecticides and the development of resistance by the insects have severely limited their application and usefulness. Insecticide-impregnated bed nets have been shown to have a significant impact on the morbidity and mortality of infection due to *P. falciparum* and *P. vivax* in China,[71] and to *P. falciparum* in Africa.[72] Malaria control schemes based on genetic modification of the capacity of vector mosquitoes to transmit the parasite have been suggested.[73] Before such strategies are implemented, a better understanding of the mosquito-parasite relationship is required. In addition, the development of more efficient methods for introducing advantageous genes into the mosquito genome need to be developed, as well as methods for replacing vector populations in the field with populations of mosquitoes unable to transmit the parasite.[74]

The World Health Organization now recommends drug treatment of the sick child as the primary mechanism to reduce malaria related mortality.[75]A malaria vaccine remains the “holy grail” of control strategies. For over 50 years, researchers have been attempting to find antigens which could prevent infection or at least reduce morbidity and mortality. After years of sporadic advances, vaccine research was reinvigorated by the persistent work of the Nussensweigs and their colleagues, demonstrating that animals could develop immunity to infection with sporozoites, and stimulated by the development of methods for the in vitro cultivation of the asexual and sexual stages of *P. falciparum* by Trager and Jensen. The revolution of molecular biology made possible the identification of specific genes coding for specific antigens and sub-unit vaccines became possible.

There are three phases of the malaria life cycle which have been targeted by the vaccine hunters.[76,77] Vaccines directed against the pre-erythrocytic stages of the parasite are intended to prevent infection by blocking the invasion or development of sporozoites freshly injected by a feeding mosquito or the development of the parasite in the liver. Secondarily it has been suggested that even partial efficacy (the blockage of most pre-erythrocytic development) could reduce the intensity of the primary infection and be useful in concert with antigens directed against other stages. Because such vaccines may have short-term efficacy, the target population for pre-erythrocytic stage vaccines has usually been considered to be non-immune individuals moving through malarious areas, including tourists and military personnel. Even with a short life, such vaccines could be useful in areas of low transmission or in children and pregnant women in areas of high transmission.[78]

Vaccines directed against the erythrocytic (blood) stages of the parasite are not expected to induce sterile immunity and totally prevent infection. Rather, it is expected that a successful vaccine could reduce the parasite burden, eliminate most deaths and reduce morbidity. The primary target for blood stage vaccines are children and pregnant women in areas of intense transmission.[79]

Vaccines directed against the mosquito (sexual) stages of the parasite are designed to block the development of the parasite in the mosquito vector. An effective vaccine could interrupt transmission to additional victims. In combination with other antigens, a transmission-blocking component could prevent the spread of parasites resistant to other vaccines. A transmission-blocking vaccine could be used in an eradication scheme or to prevent epidemics in areas of unstable malaria transmission.[80]

An extraordinary array of candidate antigens are under investigation in animal models and field trials of

several vaccine candidates are underway. The use of molecular approaches and the technology of subunit vaccines has opened the way. Expression systems, delivery systems, adjuvants and the juggling of antigen cocktails are all receiving critical examination. A virtual "antigen of the month" scenario will become even more exciting as the *Plasmodium falciparum* genome project identifies the genetic structure of more possible candidates.

Despite often tantalizing experimental successes, a malaria vaccine available for wide application is still many years away. Over the years, the parasite has shown an extraordinary capacity to evade the human immune system, and there is no doubt it will work with equal efficiency in evading many vaccine attacks. In the meantime, integrated control strategies including reduction of vector populations, reduction of human-vector contact, and chemotherapy remain the primary method for reducing the malaria burden. However, the effectiveness of these methods cannot be expected to last indefinitely, because of the development of resistance to chemicals directed against the vector and drugs aimed at the parasite. Novel approaches and new weapons are needed to prevent further deterioration of our ability to control malaria.

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