Univ. of Mustansiriyah
College of Pharmacy
Branch of Clinical Laboratory Sciences

Medical Parasitology

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Lec. 3 The Blood Parasites

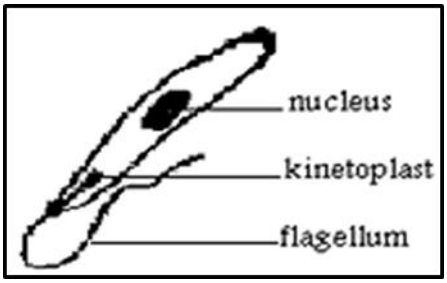
B- Blood and tissue flagellates

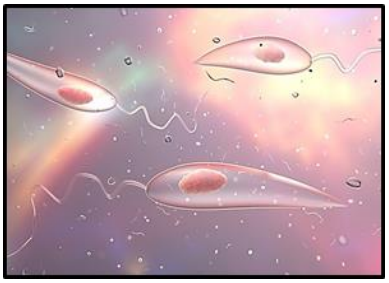
This group of flagellates also known as hemoflagellates. The term hemoflagellate indicate that the protozoan's site of residence in the human host is the blood and\or closely related tissues such as spleen and liver. They require two hosts in their life cycle: man, or another susceptible mammal on the one hand, and a blood-feeding insect (vector) on the other hand. They are belonging to two genera, ***Leishmania*** and ***Trypanosoma***.

During the life cycle of these flagellates, they may appear in four distinct morphological forms distributed between the vertebrate host (the final host) and the arthropod host (the intermediate host). It appears that any of the forms is capable of developing into any other. These forms are different in their morphology, flagellum position (and if it is present or not), the shape of the kinetoplast and its position and the presence or absence of the undulating membrane. These forms are summarized in the following:

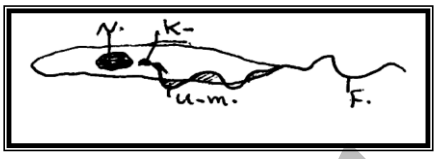
1. Promastigote (leptomonad form)

This form occurs only in the insect vector. The body is spindle in shape, the nucleus relatively in the middle of the body. The kinetoplast nearby the anterior end, the flagellum extends from this structure and become free anteriorly (extends outside the body); there is no undulating membrane in this form. Its long flagellum is serving the function of both locomotion through the medium and the attachment to the insect gut wall.



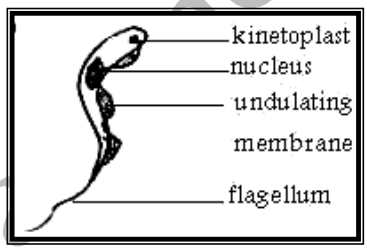


2. Epimastigote (crithidial form)



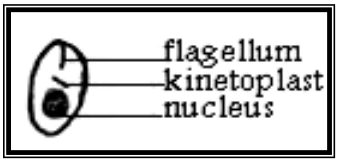
The body is spindle in shape, the kinetoplast found in front of the nucleus which is found nearby the middle of the body. The flagellum grows and extends till the anterior end, attached to the pellicle, producing an undulating membrane then extends freely.

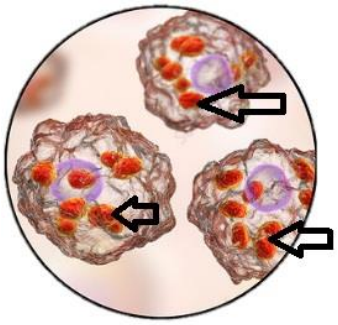
3. Trypomastigote (trypanosome form)



The body is spindle in shape, the nucleus in the middle of the body, the kinetoplast in the posterior region of the body; it produces the flagellum which extends on the out margin of the undulating membrane then extends freely.

4. Amastigote (leishmanial form)





The body is spherical or ovoid with oval nucleus found nearby the body center; in front of the nucleus, the kinetoplast is laid, which produce a short flagellum extends till the body surface, and there is no undulating membrane in this form. This form usually develops in the vertebrate host cells.

Genus *Leishmania*

Through the use of molecular and immunological techniques, a number of species and subspecies of ***Leishmania*** have been partially characterized. Of those that infect humans, three clinical manifestations are evident: **visceral**, **cutaneous**, and **mucocutaneous leishmaniasis**. While their life cycles are identical and they are morphologically indistinguishable, they differ in the type and location of the primary lesions they produce in the human host. Leishmaniasis are now endemic in 88 countries on five continents with a total of 350 million people at risk.

The vertebrate host of the parasite is the mammals, particularly the human, dogs and many species of rodents. Whereas the invertebrate host (the vector) is the sand fly.

Life cycle

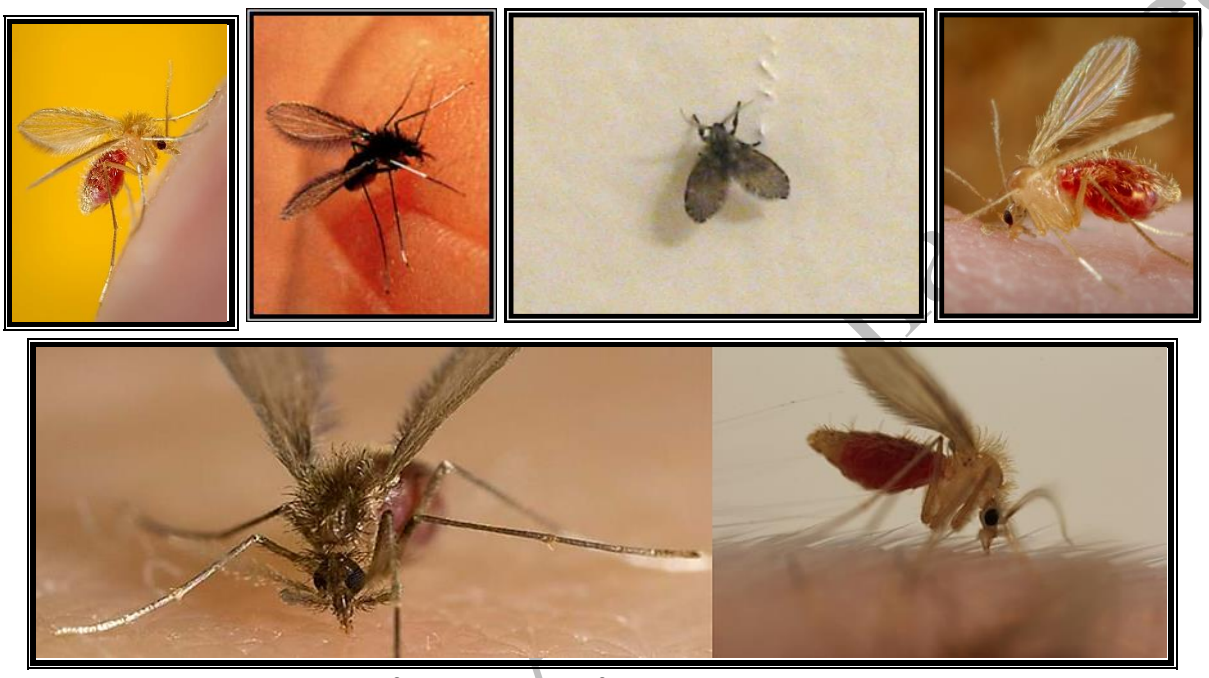
For all species of ***Leishmania***, the part of the life cycle spent in mammalian hosts is unbelievable in that the amastigote infects the macrophage, the cells of the mammalian host that constitute its primary defense against invasion by foreign organisms. The parasite, upon entering the macrophage, establishes itself in an endocytotic vacuole called a **parasitophorous vacuole**. A number of mammals act as natural reservoir hosts for the parasite, the most common being canines, both wild and domestic, and rodents. Leishmaniasis in humans is therefore a zoonosis.

In the **Vertebrate host**, when the **sand fly** takes a blood meal from man and other mammals, while they are carrying the infective stage of *Leishmania* (the **metacyclic promastigote**), it will passively inject these parasites which will be quickly engulfed by the host macrophages in the reticuloendothelial system where the **promastigotes** revert to the intracellular **amastigote** form. After sequence longitudinal binary fissions, the cells are filled with the parasites and rupture to release a large number of the amastigotes which are engulfed by other phagocytic cells, thus spreading the infection. At times, many infected phagocytic cells arrive to circulation and to viscera where the parasite will settle down and multiply in the reticuloendothelial cells of the liver, spleen and bone marrow, destroying these cells. Factors such as the species of *Leishmania* involved, temperature, immune status of the host, and even behavioral characteristics of the insect vector may determine the extent and site of infection in the mammalian host.

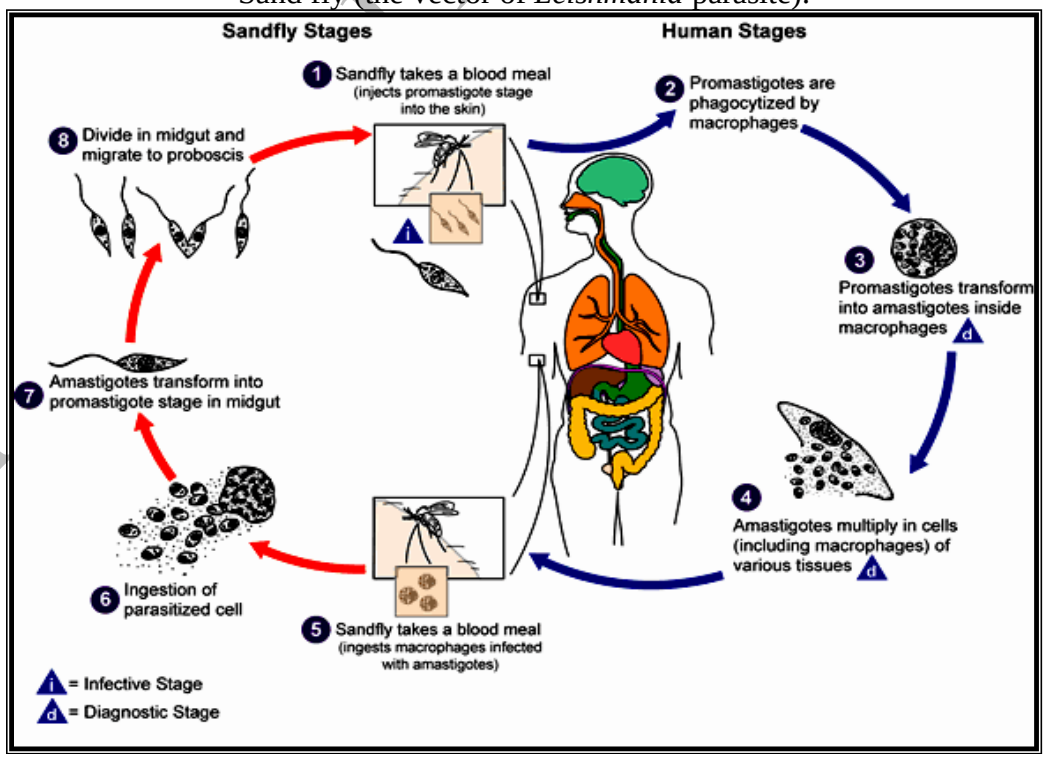
The **invertebrate host** are many species of **sand fly** from the genus ***Phlebotomus***. When the insect bites the infected vertebrate host, it will suck the blood and the amastigote stage of the parasite, which migrate to the midgut of the insect and transform to promastigote form and start in multiplication by binary fission. The parasites may be sticks on the intestinal wall of the insect or stay free in the lumen of the intestine. It may be found in aggregations of promastigote form in the first and last part of the insect gut.

After 4 to 5 days from the insect feeding, while attached to the wall of the gut, the promastigotes multiply rapidly by longitudinal binary fission and fill the esophagus. The promastigotes, as they transform to infective metacyclic promastigotes, detach from the gut wall. When they block the esophagus, the insect will push the esophagus contents to the front and back; by this method the insect will inject the infective stages in the victim which are subsequently deposited in the skin of the mammal when the sand fly feeds again.

Reservoir hosts play an important role in the prevalence of leishmaniasis. In many regions of the world, domestic reservoir hosts, such as dogs, serve as a link between the wild reservoir hosts and the human population, via the sand fly vector. The reservoir hosts are usually unaffected by the parasites; thus, they serve as a constant source of infection for the human population. Where such reservoir hosts are present, they serve as primary sources for human infection via the bite of infected sand flies.



Sand fly (the vector of *Leishmania* parasite).



"Diagram for the life cycle of *Leishmania*".

The genus ***Leishmania*** includes the following species:

1. *Leishmania donovani*

This parasite causes a disease called Kala-azar or Dum-Dum fever or visceral Leishmaniasis or black fever. It causes the most severe and often fatal form of leishmaniasis. If left untreated, it has a mortality rate of almost 100%. In the mammalian host, amastigote infected cells are found at numerous sites, e.g. spleen, liver, bone marrow, lymph glands and intestinal mucosa as well as in the endothelial cells of the blood and lymph capillaries of the spleen, liver and bone marrow. The vector of this parasite is sand fly ***Phlebotomus papatasi***. Sometimes, the parasite infects the skin again and multiplies there, in this stage the disease called **dermal Leishmaniasis** or post **Kala-azar**.

Symptoms and Pathology

Since leishmaniasis is primarily a disease of the reticulo-endothelial system macrophage, replacement of infected cells produces hyperplasia and consequent enlargement of visceral organs associated with the system, such as the spleen and liver, causes splenomegaly and hepatomegaly. A concomitant decrease in red and white blood cells production results in anemia and leukopenia, facilitating secondary bacterial infections. Without medical treatment, the condition is usually fatal. Surviving individuals, however, commonly acquire long-lasting immunity. The incubation period varies from 10 days to many months, but in the usual case, it is insidious. On the average about 90 days following exposure. The symptoms can be observed are:

1. Greatly enlargement in the spleen and the liver with an increase in size and number of parasitized Kupffer cells. In the advanced cases, enlargement of the liver and spleen produces abdominal distention.

2. The bone marrow exhibits markedly increased production of macrophages and decreased erythropoietic function.

3. Thrombocytopenia results in multiple hemorrhages, particularly from the mucous membranes.

In the typical acute case:

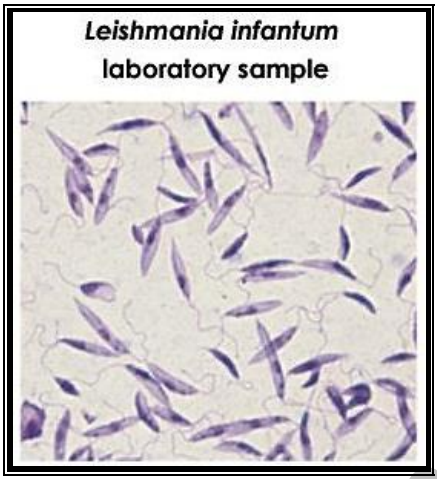
1. Temperature fluctuates daily from 37.5 - 40˚C.
2. Bleeding typically occurs from the gum, lips, naris and intestinal mucosa.

3. Complications usually observed in **kala-azar** are principally diarrhea or dysentery and broncho-pneumonia.

Diagnosis

It can be done using the serological methods (serodiagnosis) as well as the demonstration of the parasite itself microscopically in the biopsy specimens taken from the bone marrow, spleen, liver or blood sample. Definitive diagnosis based on the positive identification of intracellular amastigotes in blood or tissue smears. When such smears are inconclusive, other diagnostic techniques must be employed such as **xenodiagnosis**. The parasite found inside the macrophages.

Immunological tests are used, but these are difficult to evaluate since post-recovery cases are indistinguishable from active cases.



Treatment

Proper nursing care and complete bed rest are essential, especially in more acute cases. Chemotherapy consists of closely monitored intramuscular or intravenous injection of Pentavalent antimonial compounds such as antimony sodium gluconate. The treatment should be given until the aspirate is free of the parasites for at least 2 weeks. Extreme caution is essential in the administration of such treatment since not only can antimony produce serious side effects, but insufficient treatment may result in relapses or post-kala-azar dermal leishmaniasis. Lipid-encapsulated Amphotericin B has been shown to be successful for treatment.

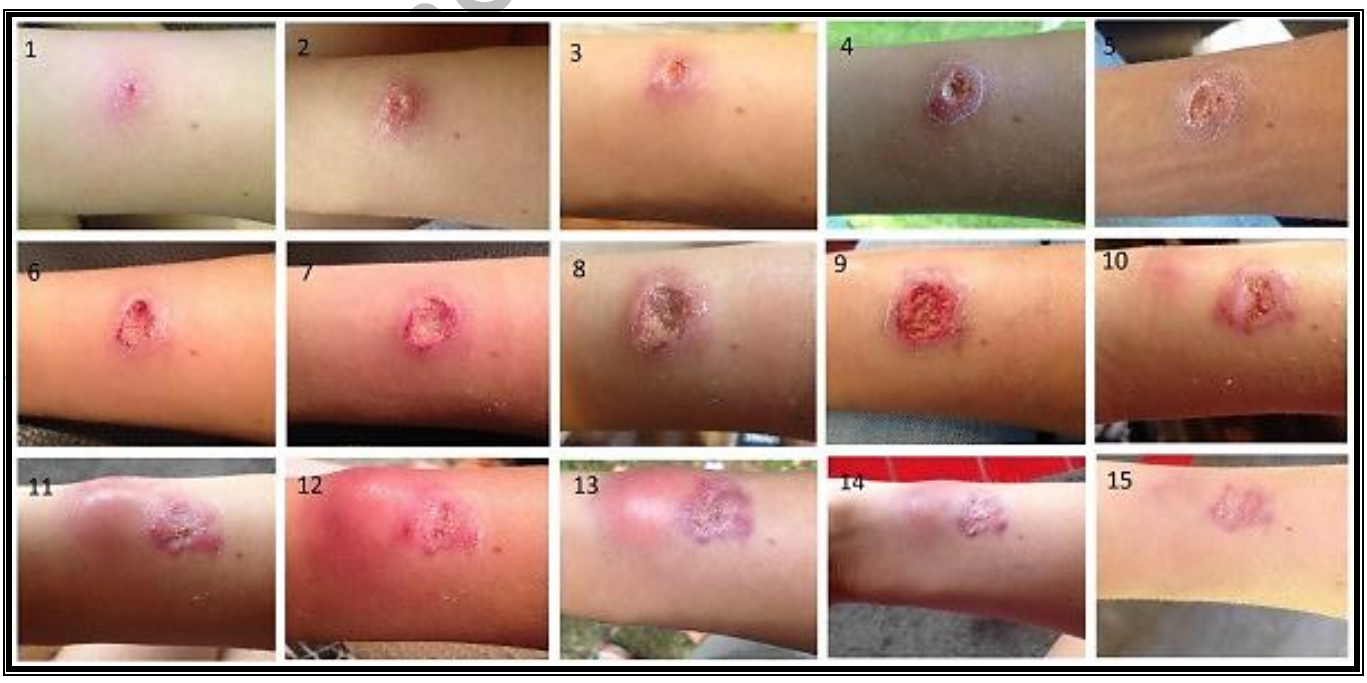
2. *Leishmania tropica*

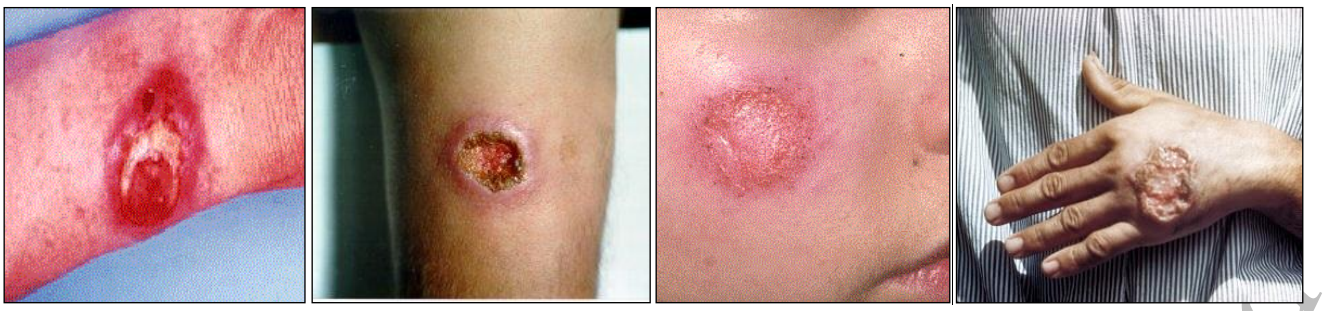
This parasite causes a relatively mild skin disease commonly known as Dry or urban cutaneous Leishmaniasis or oriental sore (or Baghdad boil or Old world cutaneous Leishmaniasis or tropica sore). The vector is ***Phlebotomus papatasi*** and ***Phlebotomus sergenti***. Unlike the amastigote of *L. donovani*, those of *L. tropica* are found primarily in the macrophages around the cutaneous sores. Sand flies must feed at these sites in order to acquire the infective amastigotes.

The tissue reaction is initiated with the introduction of the promastigotes into the dermis. The macrophages in the vicinity picks up the parasites which rapidly transform into amastigotes and multiply, destroying the host cells. Soon after, there will be a dense concentration of macrophages in the invaded area, all of which are liable to infection and destruction. The lesion then become necrotic at the center, and the margins containing parasitized macrophages which may become infiltrated with giant and plasma cells.

Pathology and Symptoms

The lesion appears in the beginning as a macula, then as a papule, with a slightly raised center covered by a thin blister-like layer of epidermis on the skin at the feeding site of the insect. The papule becomes ulcerated after a few weeks, erupts with a discharge of a small amount of clear or purulent exudates, and spreads forming cutaneous lesions most commonly on the hands, feet, legs and face.





Cutaneous lesions in cutaneous leishmaniasis.

The usual incubation period varies from 1 to 2 weeks up to several months or even, in rare instances, several years, but usually is between 2-6 months. In uncomplicated cases there are no systemic manifestations. The common occurrence of phonic complications causes painful, disfiguring, local ulcer, neutrophilic leukocytosis and fever, and at times septicemia. In the absence of secondary bacterial contamination, sores tend to heal within a year, but disfiguring scars often remain.

Diagnosis

The uncomplicated lesion may be mistaken for a variety of infections of the skin; hence, demonstration of the parasite is essential. Examination of Giemsa stained slides of the relevant tissue is still the technique most commonly used to detect the parasite. As well as the serological methods.

The most reliable diagnosis is achieved by *in vitro* culturing of lesion scrapings or aspirates and subsequent identification of the promastigotes in the medium. Antibody detection can prove useful in visceral leishmaniasis but is of limited value where most patients do not develop a significant antibody response.

Other diagnostic techniques exist that allow parasite detection and/or species identification using biochemical (isoenzymes), immunologic (immunoassays) and molecular approaches (PCR). Such techniques, however, are not readily available in general diagnostic laboratories.

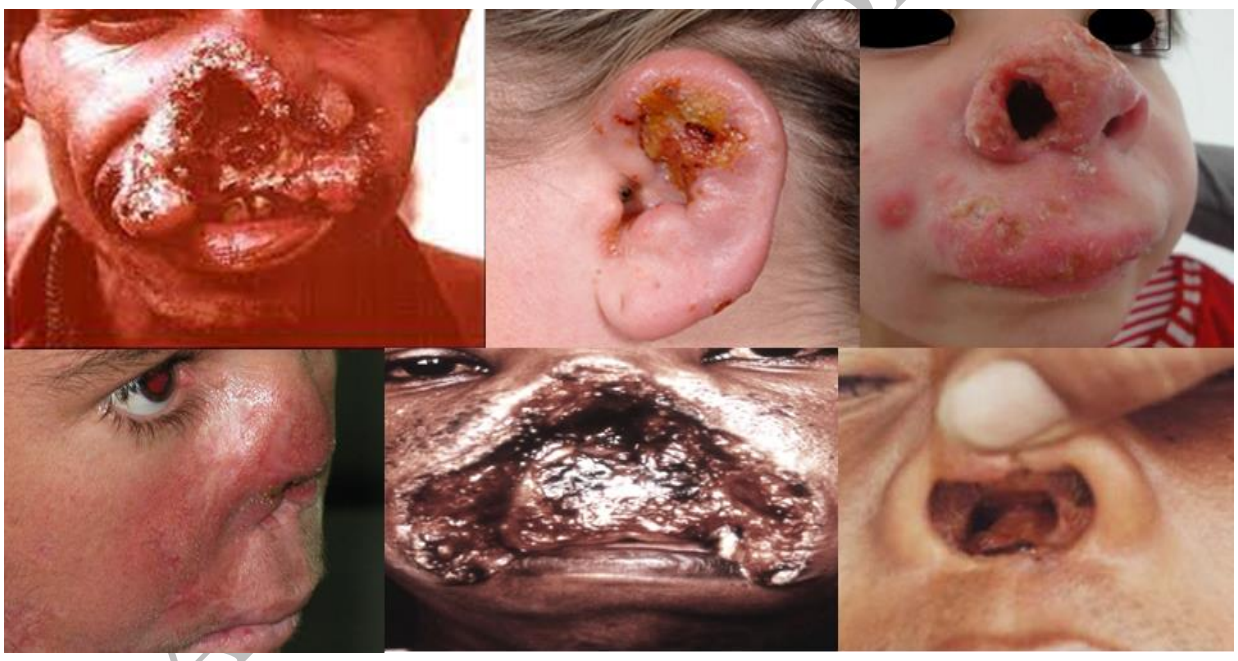
Treatment

Healing may eventually occur without chemotherapy, but the process is long and can produce disfiguring scars, especially if proper hygienic practices are not observed. Secondary microbial infections are a constant danger as long as the ulcer is open. Treatment

of choice is a daily intramuscular injection of Pentavalent antimony compounds for approximately one week. A second or third course of treatment may be required. Concomitant topical antibiotic treatment is employed in cases of microbial contamination of skin lesions.

There is another type of this disease called **mucocutaneous Leishmaniasis or Espundia** in Brazil and many other tropical parts in America. The infection clinically resembles the oriental sore, but it is caused by ***Leishmania braziliensis***. Amastigotes are found in macrophages in ulcerations at mucocutaneous junctures of the skin.

The infection starts in the skin, but the sores spreads to extent regions and appears in big numbers on the mucous membranes of the mouth, nasopharynx by extension or metastasis. It is also infecting the ear, nose cartilages and the throat, but not found in the blood and it is seldom in the inner viscera. The life cycle as similar as to that of ***L. tropica***.



Sporozoa – Malaria Parasite

Malaria is one of the most prevalent and weaken diseases afflicting humans. The disease has been reported in more than 90 countries, inhabited by 2.4 billion people. The causative agent is related to class **Sporozoa**, genus ***Plasmodium***. Although more than 50 species of *Plasmodium* infect a wide variety of animals, only four species commonly cause malaria in humans. Recently, a fifth species, ***Plasmodium knowlesi***, commonly a parasite of Old World monkeys, has been reported to infect humans in Southeast Asia.

Regardless of the species responsible for the infection, the certain aspects of the disease such as the life cycle of the infective organism, the chemotherapy and epidemiology are similar, except some medically significant dissimilarities. The four species that cause the disease in the human are:

1. ***Plasmodium vivax.***
2. ***Plasmodium falciparum.***
3. ***Plasmodium malariae.***
4. ***Plasmodium ovale.***

Malaria parasites as a group

The entire life span of the four species of ***Plasmodium*** that infect humans is spent in two hosts. The insect vector, a female mosquito belonging to the genus ***Anopheles***, and a human host. Only female mosquitoes serve as vectors; the mouthparts of males cannot penetrate human skin, hence, they feed solely on plant juices. Females, on the other hand feed on the blood which is usually required for oviposition.

The significant feature of the life cycle is the alternation of generations phenomenon, which means the life cycle includes an asexual phase (**schizogony**) alternating with sexual one (**gametogony**) followed by another asexual phase called (**sporogony**). However, in **the vertebrate host**, the asexual phase develops and gametocytes are produced. Whereas in **the vector host** (the invertebrate host), the gametocytes become mature gametes. Following maturation, the microgamete (male gametes) unites with the macrogamete (female gametes) to form the zygote, which then becomes an oocyst and produce the sporozoites. When the numerous sporozoites are introduced into the vertebrate host, they will develop in the asexual stage. Accordingly, there are two separate transfer stages, the **gametocytes** and the **sporozoites**.

The factors that influence the effectiveness of a particular species of *Anopheles* in transmitting malaria are:

1. Susceptibility to infection by the parasite.
2. Survive long enough for development and transmission of the sporozoites.
3. A preference for human blood.
4. Presence in sufficient numbers.

Anopheles mosquito (the vector)

Feeding mosquito

Life cycle

1. Schizogony (in the human)

A. Pre-erythrocytic development (Asexual Development out of the R.B.Cs.):

The inoculation occurs when an infected female ***Anopheles*** mosquito injects saliva containing sporozoites beneath the epidermis of the human victim (in the cutaneous blood vessels) in a preparation to take a blood meal, thus inoculating the sporozoites into the bloodstream. The insect injects a spindle shape bodies (the **sporozoites**) which circulate in the blood stream. After approximately 1 hour, the sporozoite disappears from the circulation, re-emerging 24 to 48 hours later in the parenchymal cells of the liver where the first colonization takes place and the exoerythrocytic schizogonic phase begins. The specificity of the relationship of the sporozoite with hepatocytes rather than with other cells of the body is due, in part, to the recognition of the surface coat of the sporozoite (circumsporozoite coat) by receptors on the surface of the hepatocytes.

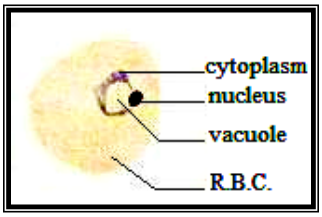
Once the sporozoite becomes inside the hepatocyte, the sporozoite develops into a trophozoite, feeding on the host cell cytoplasm. The first evidence of infection would be seen 48 hours to 7 days later in the parenchymal cells of the liver, where young schizonts in active nuclear division would be observed, and these are termed primary exoerythrocytic (EE) schizonts or pre-erythrocytic schizonts. The trophozoite will undergo two schizogony cycles, the first one called (**primary exoerythrocytic schizogony**) and the second one called (**secondary exoerythrocytic schizogony**) except in ***Plasmodium falciparum*** where the parasite undergoes single schizogony in the liver. The first generation of multiplied trophozoites called **cryptozoites** and sometimes called **merozoites**, while the second generation individuals called **metacryptozoites**. The infected liver cell with one cryptozoite produced (10-40) thousands of cryptozoites (or merozoites). After 7-10 days in the liver, the

merozoites rupture from the host cell, enter the blood circulation, and invade the red blood cells, initiating the **erythrocytic schizogony**.

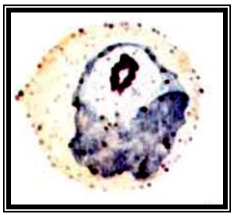
Following sporozoites-induced infection, the prepatent period for ***P. vivax*** is 8 days, for ***P. falciparum*** 6 days, for ***P. ovale*** 9 days and for ***P. malariae*** 13 days. The average incubation period (the time between inoculation of sporozoites and the first appearance of clinical sings, of which fever is the most common), is 12 days for ***P. falciparum***, 13 days to 17 days for ***P. vivax*** and ***P. ovale*** and 28 to 30 days for ***P. malariae***.

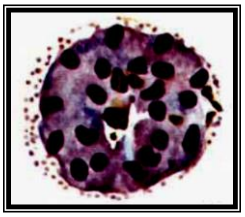
B. Erythrocytic development: (Asexual Development in R.B.Cs.):

When merozoites that have developed in pre-erythrocytic foci enters the red blood cells, they grows to the early trophozoite stage. Under the light microscopy, the early trophozoite appears to consist of a ring of cytoplasm and a dotlike nucleus. Due to its resemblance to a finger ring, this stage called the signet ring stage. In reality, the ring stage trophozoite is cup-shaped with a large vacuole filled with host hemoglobin in varying stages of digestion. This early form develops to the mature trophozoite stage. It grows because of its feeding on the R.B.Cs. contents and become rounded then irregular therefore it is called amoeboid shape trophozoite and then undergoes multiple fission and transform into schizonts later, producing a characteristic number of a new generation of merozoites in each infected erythrocyte. In the case of ***P. falciparum,*** the R.B.C. become more viscous, so it aggregates in the internal organs and does not appear in circulation. As in the liver, each of these merozoites is capable of infecting a new erythrocyte. One of two fates await this new penetrant; it may become another signet ring trophozoite and begin a new schizogony, or it may become a **male microgametocyte** (♂) or a **female macrogametocyte** (♀).These gametocytes continue in the circulation for many weeks, it isn't growing in the human body. It is important to note that these gametocytes are crescent in case of ***P. falciparum*** while they are rounded in the other types.



Ring stage.


Trophozoite.


Schizont.

It could be differentiating between the (♂ and ♀) gametocytes in the following points:

1. The microgametocyte is light blue while it is dark blue in the macrogametocyte.
2. The nucleus of the microgametocyte is large in size and has scattered granules while the nucleus of the macrogametocyte smaller and aggregated.
3. The nucleus of the microgametocyte has a light color, while it is dark in the macrogametocyte.
4. The nucleus of the microgametocyte is centric while the nucleus of the macrogametocyte is lateral or peripheral in position.

In the infected R.B.Cs. with ***P. vivax***, after the appearance of the ring stage, it will be characterized by the appearance of minute dots, stains with red color or (reddish-orange) in Giemsa or right stained thin blood films called (**Schüffner's dots**) or granules. The R.B.C. appears swollen and pale; however, these dots appears also in the case of ***P. ovale***, but in this case the infected R.B.C. becomes oval in shape (the cause of the name). In the case of ***P. falciparum***, it is show reddish dots called (**Maurer's dots**). These dots less in number and larger than the first type. In ***P. malariae*** another type of dots appears, called (**Ziemann's dots**).

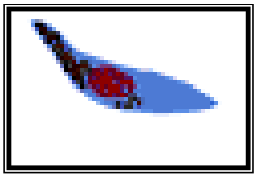
After the destruction of the R.B.Cs. the dots, many material and wastes freeing to the blood stream which originally produced by the parasites. When they reach to the spleen and the other organs or sometimes under the skin, the infected person feels with chills or shivering then fever then sweating sequently.

The merozoites spend a regular period from the entrance to the growing and forming new merozoites. This period is 48hr. in ***P. vivax*** and ***P. ovale***, 72hr. in ***P. malariae*** and 36-48hr. in ***P. falciparum***.

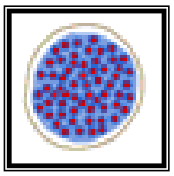
2. Gametogony (in the mosquito)

Once the ripe gametocytes are ingested by the female ***Anopheles*** mosquitoes with the blood meal and reach the midgut, they transform into mature gametes. These stages are unaffected by the digestive juices of the insect. Lysis of the surrounding erythrocytic material releases gametocytes into the lumen of the stomach. There, microgametocytes undergo a maturation process known as exflagellation during which the nucleus undergoes three mitotic divisions, producing 6 to 8 nuclei that migrate to the periphery of the gametocyte. During this period, the macrogametocytes develop into female macrogametes, each of which forms a membrane-derived fertilization cone to be penetrated by the microgamete. One macrogametocyte develops a single macrogamete. The fusion of male

and female pronuclei (syngamy) produces a diploid zygote that, after 12 to 24 hours, elongates into a motile, microscopic wormlike ookinete. This ookinete penetrates the gut wall of the mosquito to the area between the epithelium and basal lamina, where it develops into a rounded oocyst just under the outer membrane of the stomach. Following a period of growth during which its diameter increases 4 to 5 times, the oocyst is seen as a bulge on the hemocoel side of the gut.

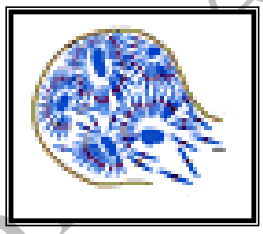


Ookinete.

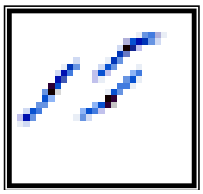


Oocyst.

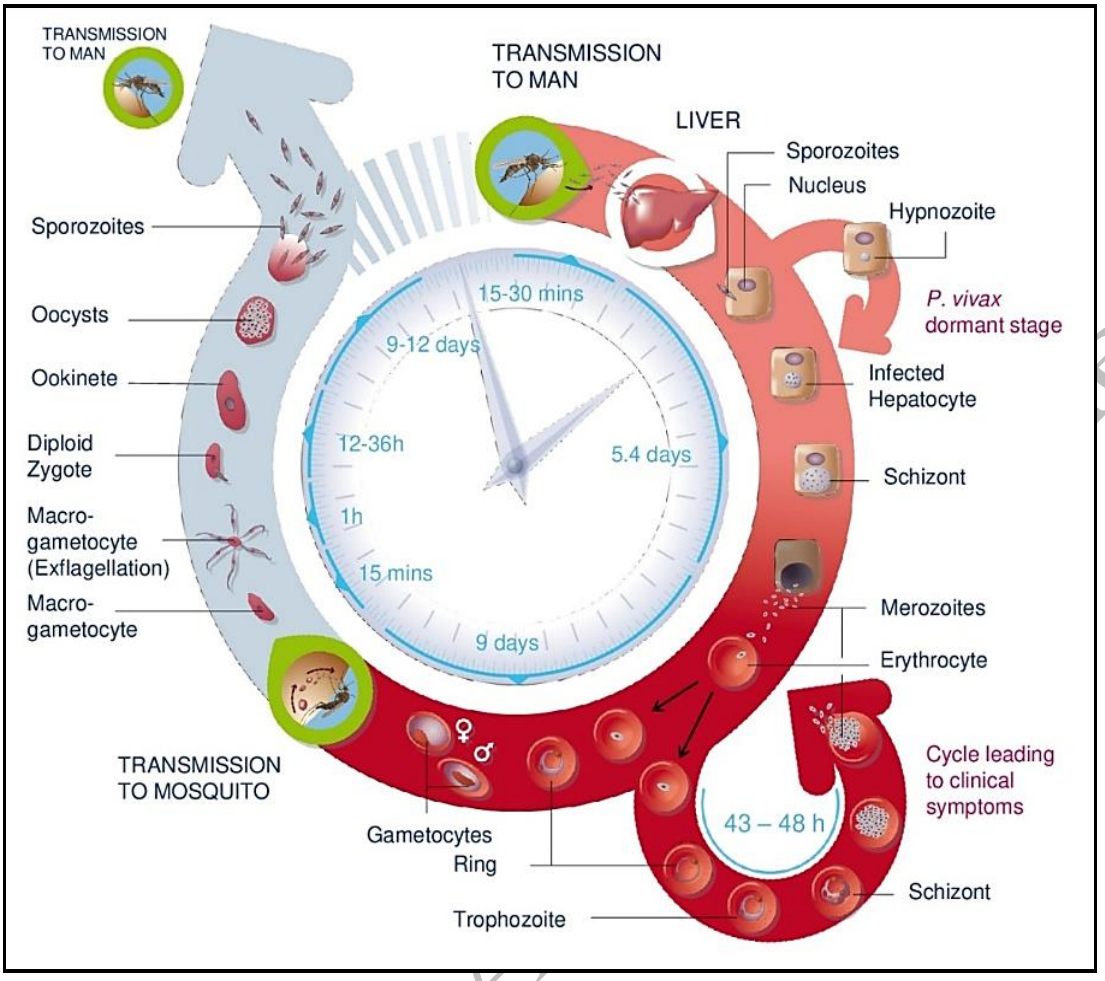
The oocyst grows rapidly and develops internal nuclear centers called sporoblasts within the oocyst. Sporoblast nuclei undergo numerous divisions, producing thousands of delicate, spindle-shaped sporozoites enclosed within the sporoblast membranes. Within 10 to 24 days after the mosquito ingests the gametocytes, the sporozoite-filled oocysts themselves rupture, releasing the sporozoites into the hemocoel. The sporozoites are carried to the salivary gland ducts of the insect and are then ready to be injected into the next victim and initiate a new infection.



Ruptured mature oocyst.



Sporozoites.



Relapse and Recrudescence

It has long been known that victims of *P. vivax* or *P. ovale* malarias, after apparent recovery, may suffer a relapse. Originally, such relapse was thought to be due to populations of cryptozoites entering the exoerythrocytic cycle. While one population progressed to the usual erythrocytic phase, the other population was thought to maintain an ongoing exoerythrocytic cycle known as a **para-erythrocytic cycle**.

It was believed that parasites in the hepatic stages of the cycle remained protected from host antibodies until activated by some physiological change within the host that allowed them to erupt from the hepatocytes, precipitating another bout of malaria. Now, it is recognized that there are two different populations of sporozoites. Short prepatent sporozoites (SPPs), upon entering the human host, undergo the usual exoerythrocytic phases of development and cause malaria. Long prepatent sporozoites (LPPs) or **hypnozoites**, remain dormant in the hepatocytes for an indefinite period. When a stimulus, such as the

physiological fluctuation cited above, activates hypnozoites into the exoerythrocytic and erythrocytic cycles, relapse occurs.

Symptomatology

Pathology in human malaria is generally manifested in two basic forms: host inflammatory reactions and anemia. Of the four species of *Plasmodium* responsible for human malaria, *P. falciparum* is the most virulent and causes, by far, the highest mortality.

The initial symptoms of malaria, such as nausea, fatigue, a slight rise in temperature, mild diarrhea and muscular pains, are often mistaken for influenza or gastrointestinal infection. Host inflammatory reactions are triggered by the periodic rupture of infected erythrocytes, which releases malarial pigment such as hemozoin **(hemozoin is a disposal product formed from the digestion of blood by some blood-feeding parasites)**, cellular debris and parasite metabolic wastes into the circulatory system. Macrophages, particularly those in the liver, bone marrow and spleen, phagocytose released pigment. In extreme cases of *P. falciparum* malaria, the amount of pigment is so great that it imparts a dark, reddish-brown hue to visceral organs such as liver, spleen and brain. With increased erythrocyte destruction, accompanied by the body's inability to recycle iron bound in the insoluble hemozoin, anemia develops.

The incubation period extends from many weeks to months until the symptoms appear, which is sequent paroxysm in regular periods of shivering or chills then fever then sweating; the chills extend for 5-15 min., while the fever from 1-2hrs., and sweating for many hours.

Other important symptoms characterized by splenomegaly, hepatomegaly and increase in the bone marrow activity; there is also secondary sings like constipation, diarrhea and anemia (pernicious anemia), and sometimes relapses occur after the disappearance of the symptoms when the immune system become non-efficient. Whenever, the symptoms of the infection would appear without new exposure for mosquito bite, the cause of that is some hidden stages in the liver cells.

A condition known as **blackwater fever** often accompanies *P. falciparum* malaria, characterized by massive lysis of erythrocytes. It produces abnormally high levels of hemoglobin in urine and blood, fever, vomiting with blood, and jaundice, and there is a 20 to 50% mortality rate usually due to renal failure. The exact cause of this condition is uncertain; it may be a reaction to **quinine**, or it may result from an autoimmune phenomenon in which hemolytic antibodies are produced.

Host Immune Response

The immune response of the human host differs somewhat for each of the two stages in the malarial life cycle i.e., the pre-erythrocytic stage and erythrocytic stage. It is believed that T-cells, notably $CD8^+$ T cells, play an important role in pre-erythrocytic immunity. On the other hand, $CD4^+$ T cell regulation appears to play a critical role in acquired immunity to the erythrocytic stage of malaria infection.

Diagnosis

By making (Blood films):

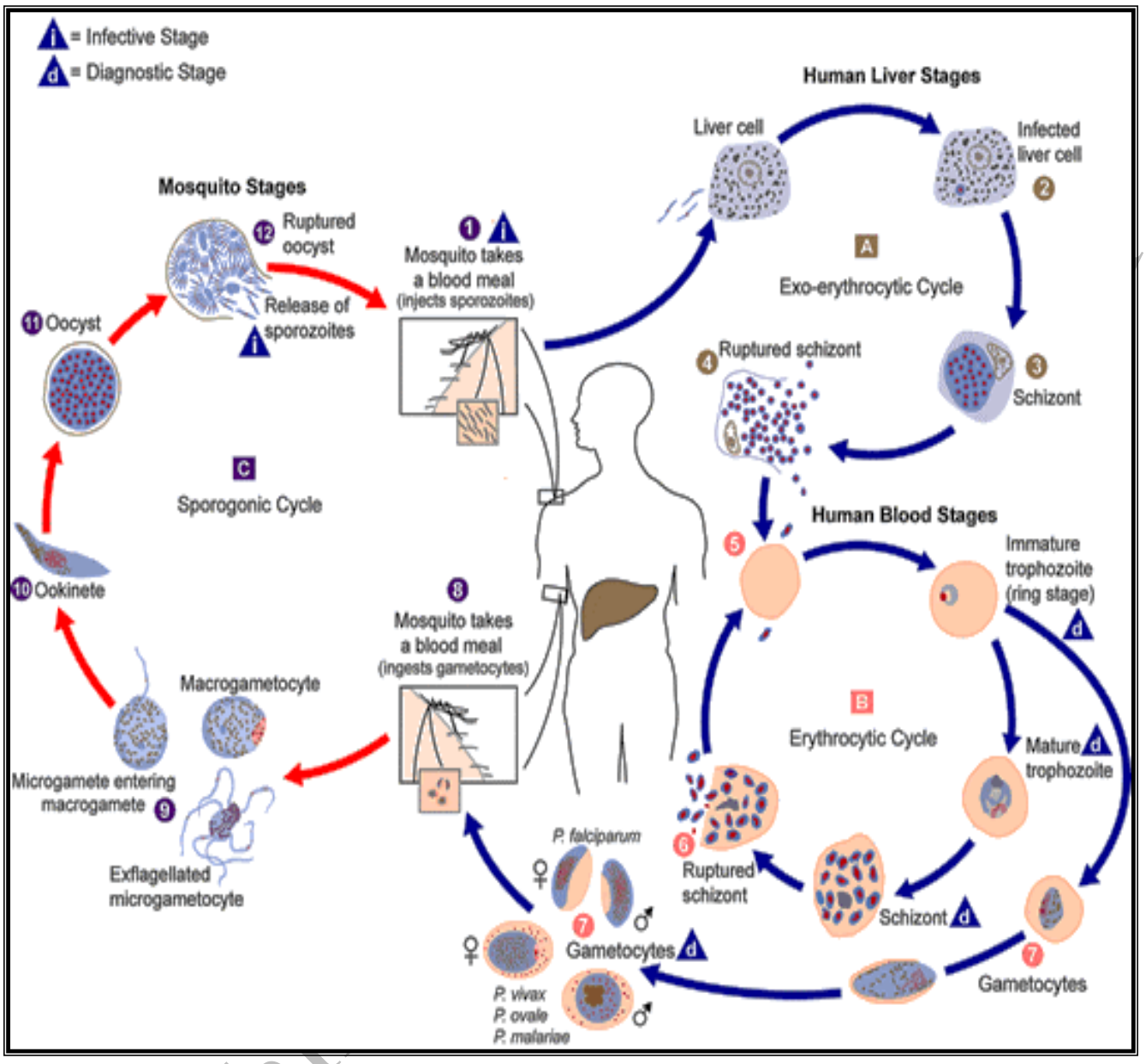
1. Thick blood films: are frequently necessary to detect the parasites. This type allows rapid examination of a large volume of blood in a small area on the slide. Staining of thick blood films is carried out according to (Giemsa technique). These films provide concentration of the parasites.

2. Thin blood films: it is also essential that thin films be prepared because the malarial species can be more readily identified on these, especially less experienced examiners. It is stain by Giemsa stain or Right stain.

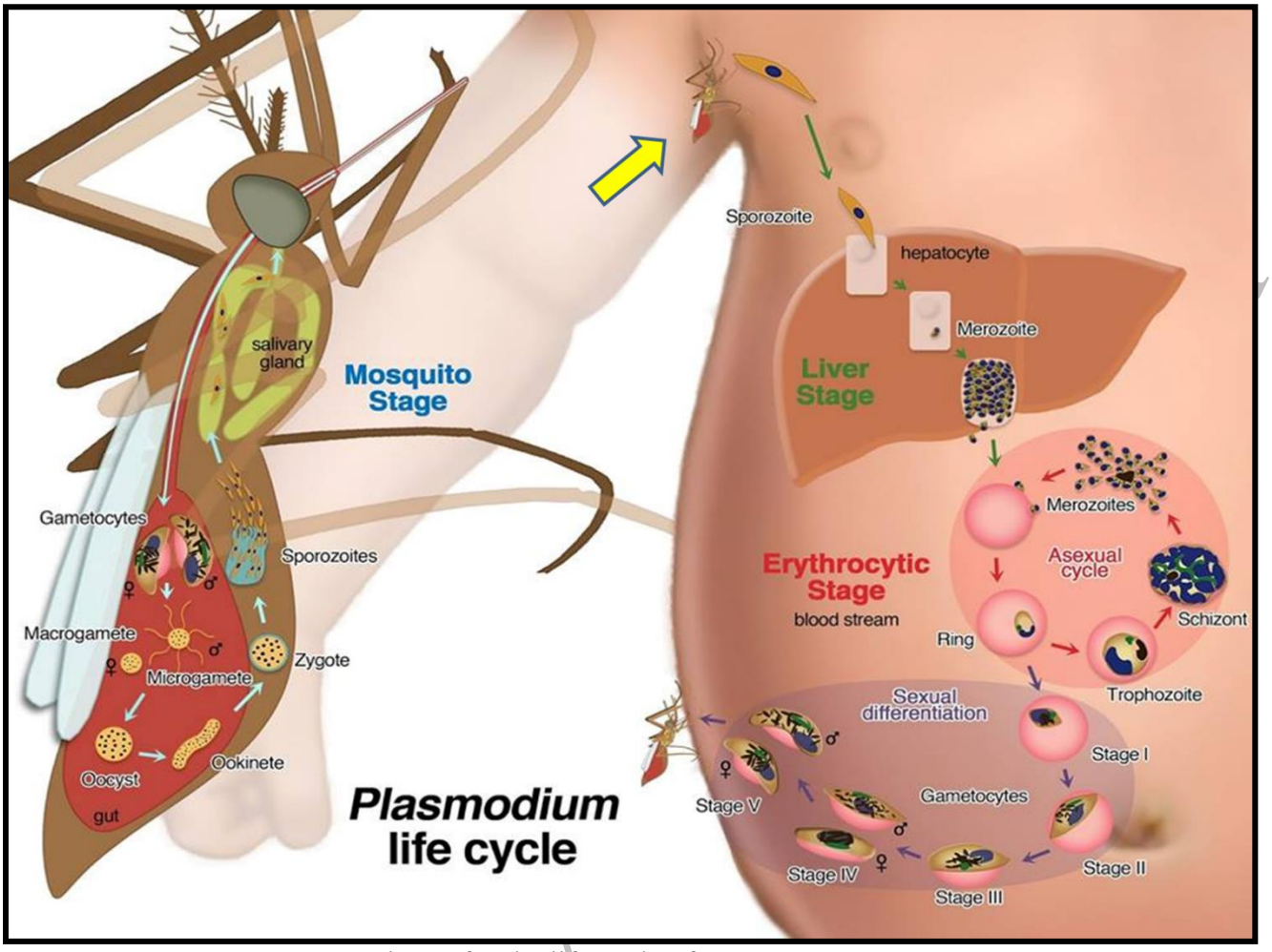
Treatment

1. All malaria infection except resistant *P. falciparum*: Chloroquine diphosphate (orally).
2. Treatment of attack:

If oral dose can't be given, Quinine dihydrochloride (IV), Chloroquine hydrochloride (IM).



"Diagram for the life cycle of *Plasmodium* sp."



"Scheme for the life cycle of *Plasmodium* sp.".