Tikrit University
College of Pharmacy

Microbiology II
-Medical Parasitology

MEDICAL PARASITOLOGY

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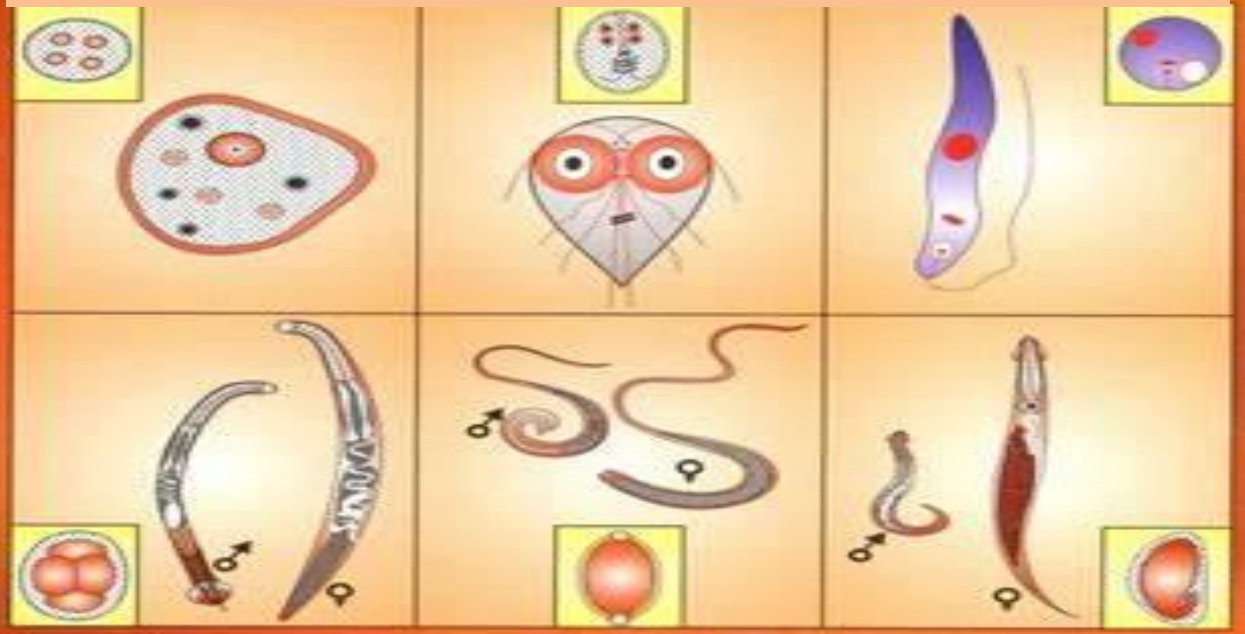

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Trypanosomes

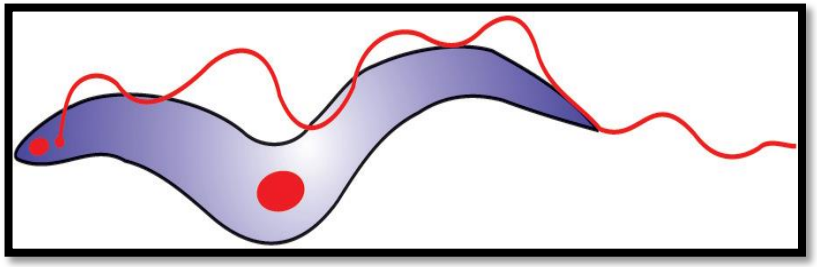
Trypanosomes are haemoflagellates that live in the blood and tissues of their human hosts. *Trypanosoma brucei* has three subspecies *Trypanosoma brucei brucei*, *T. b. gambiense* and *T. b. rhodesiense*. The first one is an animal pathogen and the other two cause African trypanosomiasis or sleeping sickness in man. Whereas *Trypanosoma cruzi* causes South American trypanosomiasis or Chagas' disease. Forde, 1902 was the first to demonstrate motile trypanosomes in the blood of a man suffering from fever. This parasite was subsequently named as *T. gambiense* by Dutton in 1902. Kleine, 1909 demonstrated the development of the parasite in the vector, the tsetse fly.

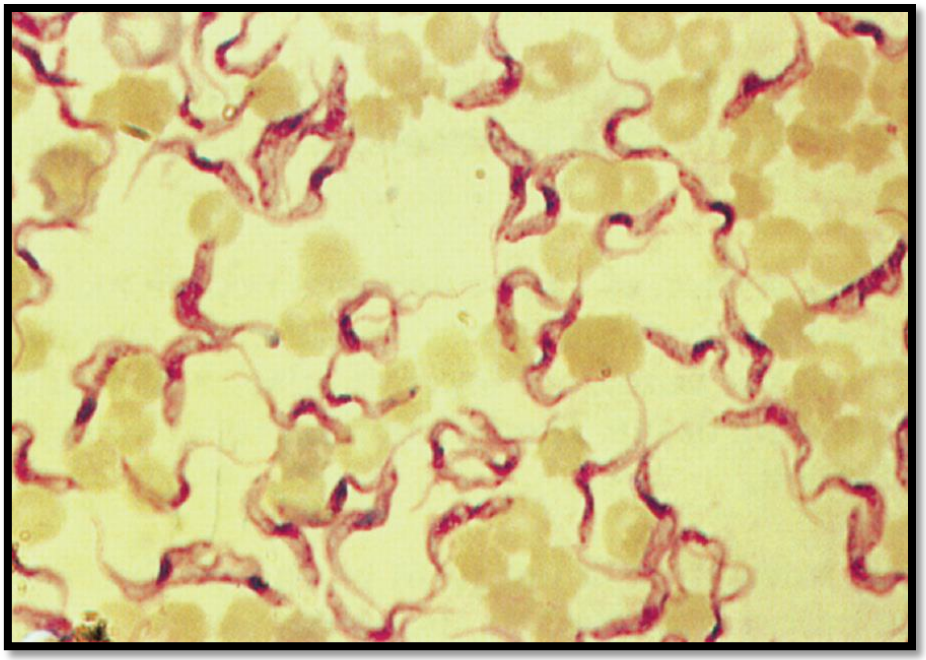
Trypanosoma brucei gambiense

It occurs in West and Central Africa; it inhabits the connective tissue spaces of the various organs, the reticular tissue of the lymph nodes and spleen, the intercellular spaces in the brain, the lymph channels throughout the body, blood and cerebrospinal fluid. In the blood of the vertebrate host, *T. b. gambiense* exists in trypomastigote form.

In fresh blood the trypanosomes may be seen as motile, colorless, spindle-shaped bodies with a blunted posterior end and a finely pointed anterior end. The nucleus is large, oval and central in position. Kinetoplast is situated on the posterior end. From that area the flagellum arises. It curves around the body in the form of an undulating membrane and then continues beyond the anterior end as a free flagellum.

In Giemsa or Wright stained preparations, the cytoplasm and the undulating membrane appear pale blue, the nucleus reddish-purple or red and the kinetoplast and the flagellum dark red.





Trypanosoma brucei gambiense.

One of the most important aspects of the trypanosomes is their ability to vary the antigenic nature of their surface coat. It appears that during the growth of trypanosomes new variant antigen types (VAT) are constantly being produced by mutations, additions, deletions and recombination.

Each wave of parasitemia caused by a new variant emerges at 5-10 days' interval and is accompanied by fever and followed by monocytosis and the production of antibodies against the variant surface glycoproteins (VSG). This variant-specific antibody is involved in the removal or clearance of the majority of the trypanosomes from the blood and other body fluids, the process that ends each wave of parasitemia.

Life cycle

This parasite passes its life cycle in two hosts. The vertebrate hosts are the man and domestic animals, and the invertebrate host is the **tsetse fly** of the genus ***Glossina*** (***G. palpalis***). Both male and female flies bite man and may serve as vectors.

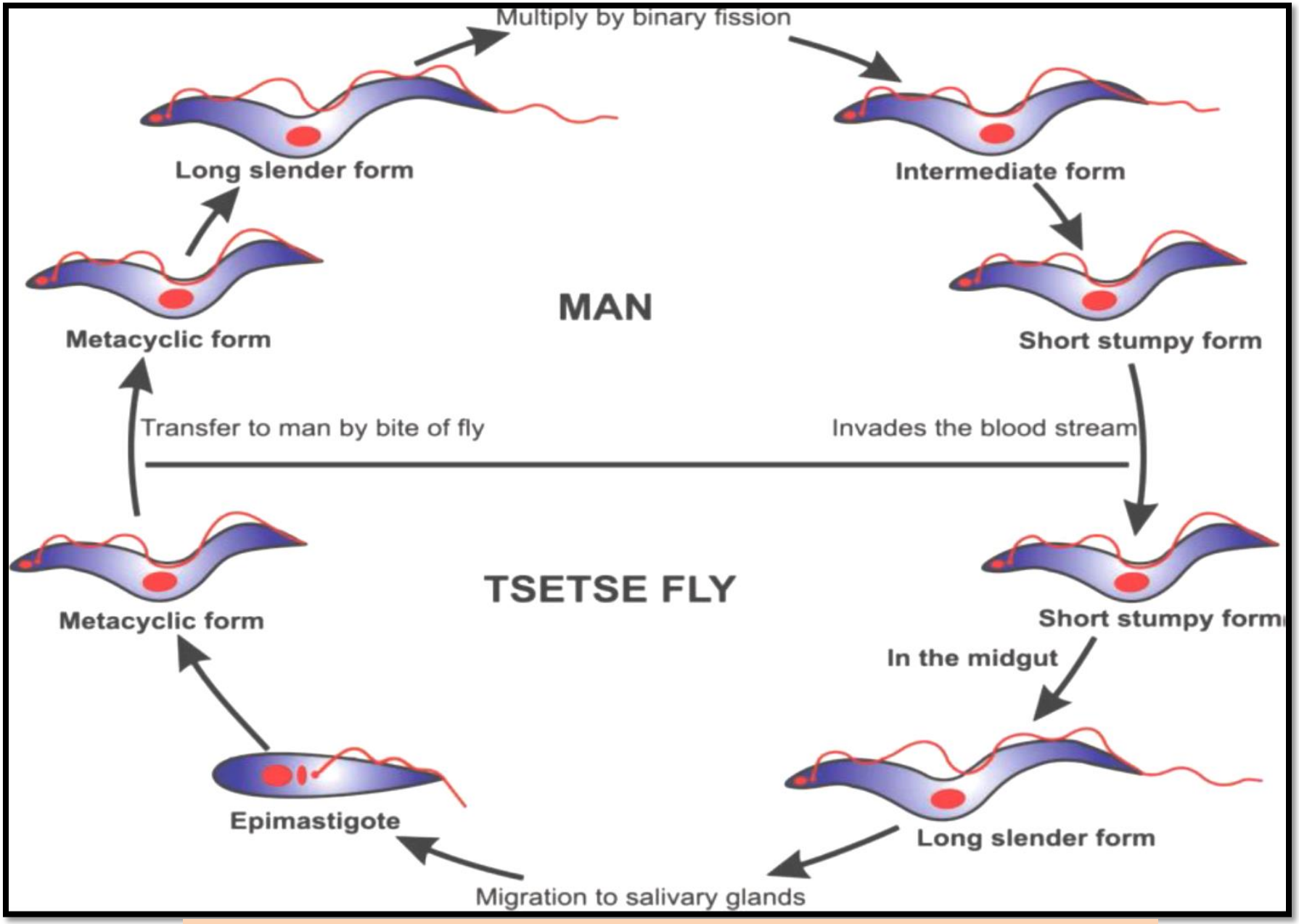
Development in man and other vertebrate hosts

During its feeding, the tsetse fly (*Glossina* spp.) introduces the metacyclic trypomastigotes into the mammalian host in saliva injected into the puncture wound. They transform into long slender forms and multiply by binary fission at the site of inoculation then transform first into an intermediate stage and then into a non-dividing short stumpy form with no free flagellum. Subsequently, the parasites invade the blood stream, resulting in parasitemia.

The trypomastigote forms, particularly the short stumpy forms, are taken up by the tsetse fly along with its blood meal. It has been suggested that the short stumpy stage is the infective stage for the tsetse fly. The transition from the long slender form to the short stumpy form may, therefore, be critical for the transmission of infection to the fly and the successful completion of the life cycle.

When an uninfected tsetse fly bites an infected vertebrate host the development in the vector is initiated. In the midgut of the insect, the short stumpy forms develop into long slender forms and multiply. These forms then pass to the posterior end of the insect where they continue to multiply for some days. By the 15th day, they escape and migrate forwards and gain access to the salivary glands via the hypopharynx and salivary ducts.

In the salivary glands they develop into epimastigotes and attach to the cells of the glands. In the epimastigote forms, the nucleus is posterior to the kinetoplast, in contrast to the trypomastigote, in which the nucleus is anterior to the kinetoplast. They divide repeatedly and then transform into non-dividing metacyclic forms, which are highly motile, short and stumpy. When mature the metacyclic forms detach from the salivary gland cells and are infective to the vertebrate host. The time taken for the complete evolution of the infective metacyclic forms inside the tsetse fly is about 20 days.



“Diagram for the life cycle of African Trypanosomiasis”.

Pathogenicity

This parasite causes **African trypanosomiasis** (**West African sleeping sickness**). It is chronic in nature lasting up to 4 years. The chronic nature of the disease can be attributed to antigenic variation in which waves of parasitemia occur in the blood of the infected person. The insect bite during the day, usually in the early morning and evening. Metacyclic forms are injected into the subcutaneous tissue of a man at the time of the bite. Some of the parasites may enter directly into the blood stream but the majority of them multiply locally. Then the trypomastigotes spread throughout the entire body. They move through the blood and lymphatic vessels and multiply rapidly.

A chancre (3-4 cm) develops at the site of the bite. It is a hard and painful nodule and fluid withdrawn from it contains actively dividing trypomastigotes. It resolves spontaneously within 1-2 weeks. Patients first experience intermittent recurring fever associated with lymphadenopathy. Lymph nodes in the posterior cervical region of the neck are frequently involved, producing a lesion known as **Winterbottom's sign**.

Hepatosplenomegaly may also be evident during the early stage of infection. If untreated, the central nervous system is involved. Trypomastigotes enter the subarachnoid space and then the brain substance, with infiltration of plasma cells and lymphocytes, and perivascular proliferation of endothelial and neuroglial cells between the blood vessels and perivascular sheath.

At this stage, the patient develops severe headache and a wide array of behavioral changes ranging from aggressiveness to sleep-like states. Sleepiness becomes so pronounced that the patient falls asleep while eating, standing or sitting (sleeping sickness) and dies.

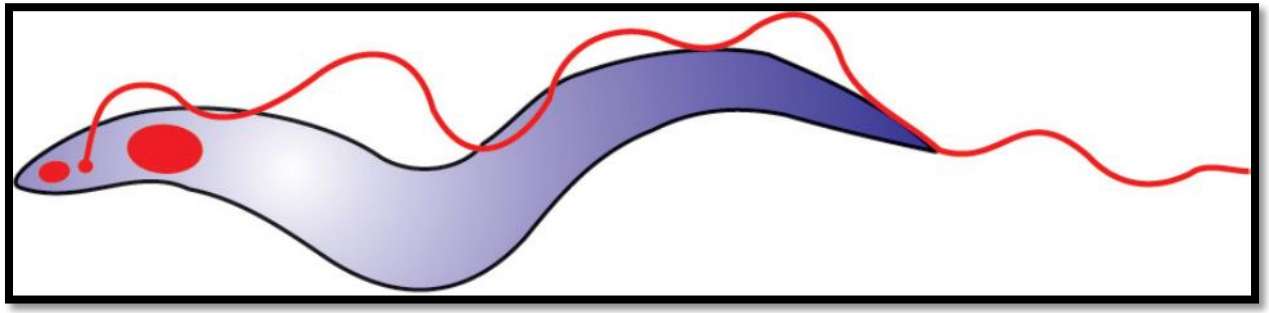
Diagnosis

The diagnosis of African trypanosomiasis depends upon the demonstration of the parasite (trypomastigote forms) in blood, lymph node aspirates, sternal bone marrow, cerebrospinal fluid or fluid aspirated from the trypanosomal chancre using direct microscopic examination of unstained and stained films.

Trypanosomes in various clinical specimens can also be detected by cultivation in NNN medium. A number of serological tests are also available; these include the indirect fluorescent antibody test, indirect haemagglutination test, ELISA can be used to detect antigens in serum and CSF, and card agglutination test for trypanosomes (CATT). These tests detect antibodies in the sera of infected individuals and utilize antigens from blood stage trypanosomes.

Trypanosoma brucei rhodesiense

It occurs in East Africa. It was discovered by Stephans and Fanthan in 1910 in the blood of a patient in Rhodesia with symptoms consistent with “sleeping sickness”. The habitat, antigenic variations and morphology of *T. b. rhodesiense*, both in man and in transmitting flies, are identical to those of *T. b. gambiense*. Like *T. b. gambiense*, it can also be cultured on NNN medium. Its life cycle is also similar to that of *T. b. gambiense*. However, the principal insect vectors are ***G. morsitans, G. pallidipes*** **and** ***G. swynnertoni***. Antelopes and possibly other wild game and domestic cattle are reservoir hosts.



Posterior nucleate form of Trypanosoma brucei rhodesiense.

The disease produced by *T. b. rhodesiense* is called **East African sleeping sickness**, it is similar to that of *T. b.gambiense*. However, there are differences in the clinical manifestations of the disease they cause. The major difference is that the disease produced by *T. b. gambiense* is chronic in nature lasting up to 4 years, whereas the disease produced by *T. b. rhodesiense* is more acute, rarely lasting more than 9 months before death occurs.

In *T. b. rhodesiense* infection, febrile paroxysms are more frequent and oedema, weakness, rapid loss of weight, myocarditis and fever are striking symptoms, but lymph node enlargement is less pronounced. Mania and delusions are often noted, but the profound somnolence (drowsiness) and other marked nervous symptoms of the sleeping sickness stage are lacking or not so evident in this infection.

T. b. rhodesiense is more resistant to treatment in the advanced stage of the disease. Laboratory diagnosis, treatment and prophylaxis are as same as for *T.b. gambiense* infection.

Treatment

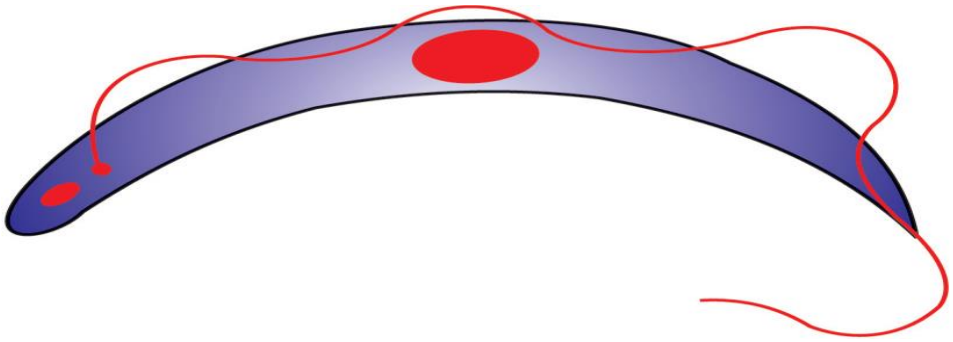
Suramin is used to treat patients with primary stage infections that do not involve the CNS. Because it does not cross the blood-brain barrier it is not effective against the secondary CNS stage. It is relatively toxic und many cause optic atrophy, blindness, nephrotoxicity and adrenal insufficiency in some patients. Pentamidine isethionate, like suramin, does not cross the blood-brain barrier, therefore, it can also be used in the initial stages. It may produce nephrotoxicity, hepatotoxicity and pancreatic toxicity in some patients. Melarsoprol can cross the blood-brain barrier and is used to treat patients in the late secondary CNS stages of trypanosomiasis.

Trypanosoma cruzi

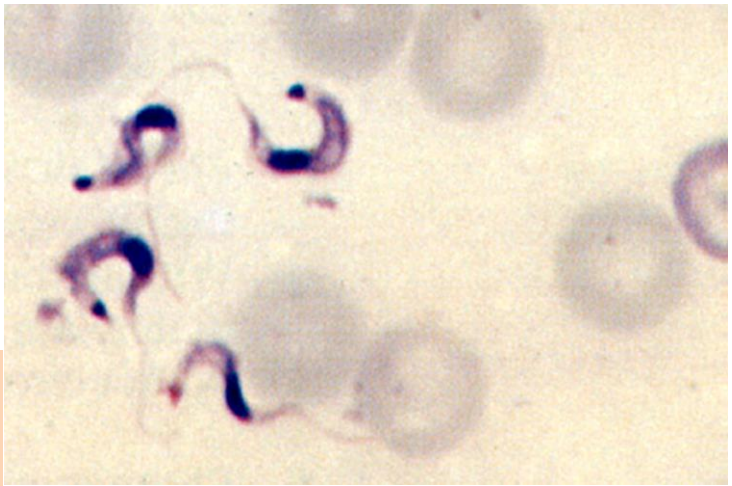
It occurs in Central and South America. Carlos Chagas, investigating malaria in Brazil in 1909, accidentally found this trypanosome in the feces of a reduviid bug and in the blood of a monkey bitten by the infected bugs. It was only later that Chagas found the trypanosome in the blood of a sick child and showed that it was responsible for an endemic disease which came to be named after him. Chagas named the parasite *T. cruzi* after his mentor Oswaldo Cruz.

It lives as a trypomastigote in the blood and as an amastigote in reticuloendothelial cells and other tissue cells of man and many mammals. In man, the most frequent locations of the parasites are reticuloendothelial cells of the spleen, liver, lymph nodes, bone marrow and myocardium.

Parasites may also occur in cells of striated muscles, nervous system, histiocytes of cutaneous tissue, cells of the epidermis and in the intestinal mucous membrane. The trypomastigotes are present in the blood of the patient during the early acute stage and at intervals, thereafter in smaller numbers. In stained preparations, the parasite shows a characteristic C-shape. It measures 20 μm in length and 2-4μm in width, has a central nucleus and a large kinetoplast situated at the posterior end. Two forms occur in the blood, a long slender one and a short broad one.



Trypomastigote form of Trypanosoma cruzi.



The amastigotes are round or oval in shape, measure 1.5-4.0μm in diameter, have a large nucleus and a kinetoplast. In fixed and wandering (travelling) histiocytes, especially in the spleen, liver, lymph nodes and bone marrow, the amastigote is indistinguishable from that of *L. donovani*.

In myocardium and neuroglial cells, the amastigote forms are collected within a cyst-like cavity in the invaded cells. Staining reaction is the same as that of other trypanosomes. It can be easily cultivated in the epimastigote form in NNN medium

Life cycle

T. cruzi passes its life cycle in two hosts, man or the reservoir hosts such as armadillos, opossums, wood rats and raccoons. The other host is the bloodsucking insect, the reduviid bug (***Triatoma infestans***).

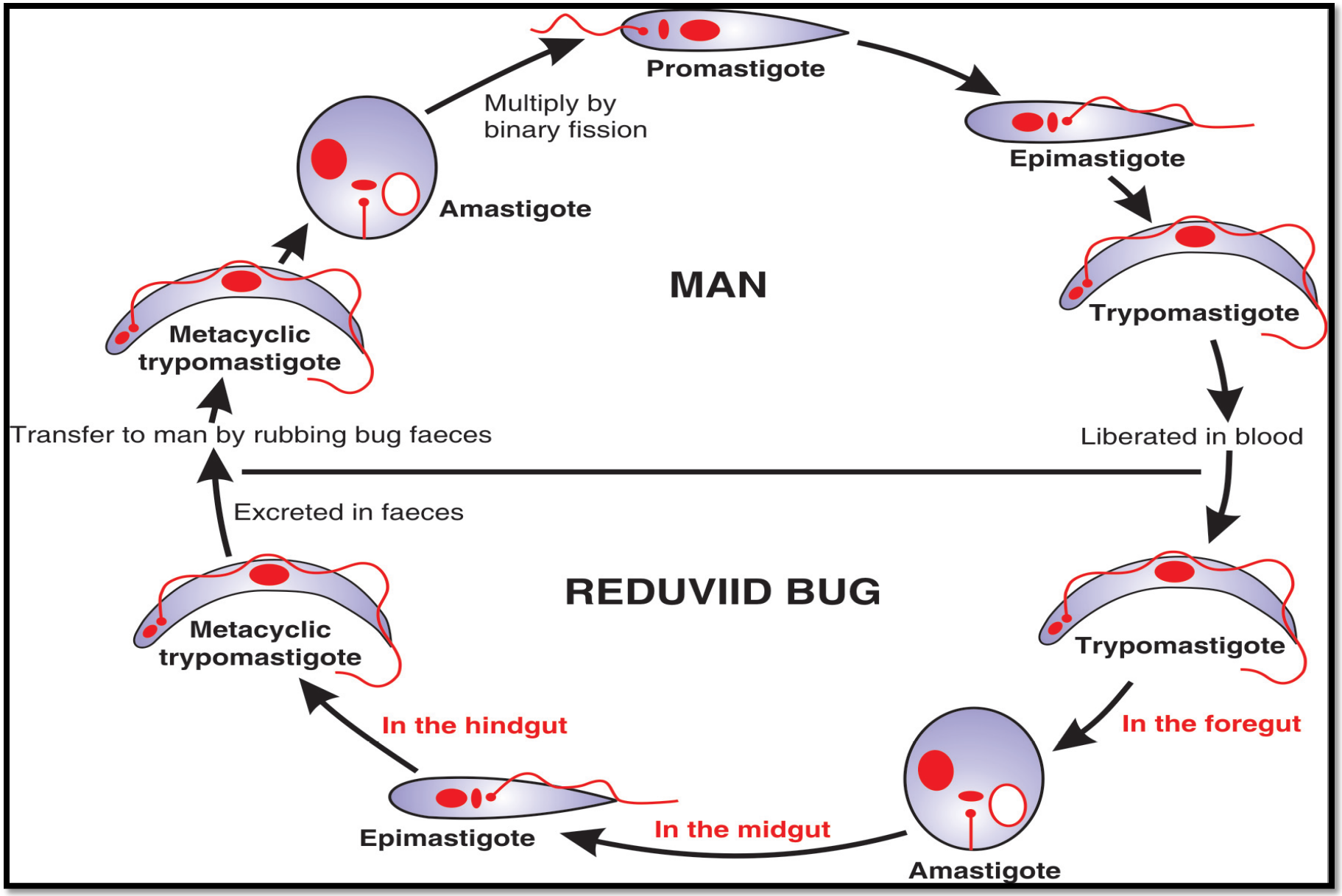
Development in reduviid bug

Bugs acquire infection by feeding on an infected mammalian host. Trypomastigotes in the blood meal transform into amastigotes in the foregut and multiply by binary fission. In the midgut, they divide by binary fission in the epimastigote stage. In the hindgut, epimastigotes attach to the epithelium, transform into **metacyclic (infective) trypomastigotes** and are excreted in the feces of the bug. The development of *T. cruzi* in the vector takes around 10-15 days.

Development in man

The infective forms of *T. cruzi* are found in the bug's feces. The bugs tend to defecate very soon after taking a blood meal. Since the bug's saliva contains an irritant, the person tends to scratch, thus scratching in the infective forms from the bug's feces. Most reduviid bugs are nocturnal and feed on sleeping inhabitants of the house.

They are attracted to the host by warmth, carbon dioxide and odor. The infection can also be transmitted by contamination of abraded (scratched) skin and the conjunctiva, blood transfusion, organ transplantation, placental transfer, and accidental ingestion of parasitized reduviid bugs.



Life cycle of Trypanosoma cruzi.

The metacyclic trypomastigotes thus introduced, invade cells of the reticuloendothelial system and other tissues particularly muscle and nervous tissue and are transformed into amastigote form. These multiply by binary fission and after passing through promastigote and epimastigote forms they are again transformed into trypomastigote forms which are liberated in the blood. This leads to the dissemination of infection and infects fresh reduviid bugs when they next feed.

Pathogenicity

T. cruzi causes **South American trypanosomiasis** or **Chagas' disease**, a zoonotic disease that can be transmitted to humans by blood-sucking reduviid bug. The incubation periodmay be as short as two weeks, or several months if the infection is acquired by blood transfusion.

The prolonged incubation period in recipients given contaminated blood is thought to be due to the poor capacity of circulating broad blood forms to invade cells. At the site of entry of *T. cruzi* a subcutaneous inflammatory nodule develops. It is known as **chagoma**. Rarely multiple chagomas have been described.

When the entry is through the conjunctiva, the patient develops painless, inflamed, periophthalmic, unilateral oedema and conjunctivitis. It is known as **Romana's sign**. The primary lesion is accompanied by fever, acute regional lymphadenitis and dissemination to blood and tissues. The parasites can usually be detected within 1-2 weeks as trypomastigotes in the blood. Acute infections are more common and more severe in children.

About 10% of children die during the acute stage. At this stage patient develops a fever, hepatosplenomegaly, generalized lymphadenopathy, facial or generalized oedema, rash, vomiting, diarrhoea, anorexia and ECG changes like sinus tachycardia and the patient may die of acute myocarditis.

It may also lead to meningoencephalitis mainly in infants and AIDS patients. Patients surviving the acute infection develop a chronic disease in which cardiac changes are most common with arrhythmias, palpitations, chest pain, oedema, dizziness, syncope (fainting) and dyspnea.

The patient may also develop dilatation of the esophagus (megaesophagus) and colon (megacolon), loss of peristalsis, regurgitation, dysphagia and severe constipation. Congenital transmission can occur in both the acute and chronic stages of the disease.

Common clinical findings of congenital infection are stillbirth, low birth weight, myocarditis, neurologic alterations, and death shortly after birth. Infants of seropositive mothers should be monitored for up to 1 year after birth. Monitoring should include an examination of blood for parasites and serologic tests.

Immune response

Shortly after infection, humans develop an immune response to the presence of the parasite. Both antibody-mediated and cell-mediated mechanisms are involved. During the initial phase of infection, IgM is predominant, whereas later, IgG and IgA become major antibody classes. Antigenic variation, which is characteristic of infections with African trypanosomes, is less common in *T. cruzi* infection.

In spite of humoral and cellular immunity, the infection is able to persist in the host. Cell-mediated immunity has also been implicated as a cause of tissue destruction, including cardiomyopathy and megacolon, seen

in Chagas' disease.

Diagnosis

The definitive diagnosis depends on the demonstration of the trypomastigotes in the blood, amastigote stages in tissues, positive PCR and serological tests. For microscopic examination, aspirates from the chagomas and enlarged lymph nodes can be examined for amastigotes and trypomastigotes. In acute infection, *T. cruzi* may be found transiently in the peripheral blood, by direct microscopy of Giemsa stained and unstained wet blood film detection of the parasite is performed.

In chronic disease, the trypomastigote stage is rare or absent, except during febrile episodes. Laboratory workers should use bloodborne pathogen precautions when examining blood films from Chagas' disease patients because the trypomastigotes are infective.

Blood and other specimens can be cultured by inoculation in NNN medium and incubated at 25°C. The PCR may be very useful for the diagnosis of patients with chronic Chagas' disease because of the lack of sensitivity and specificity of serologic tests and xenodiagnosis. Serodiagnosis detects exposure to infection rather than an active infection. Antibodies against *T. cruzi* may be detected in the patient's serum by complement fixation test, indirect fluorescent antibody test, ELISA and IHA test.

In *T. cruzi* infection, seropositivity is generally maintained for life unless specific anti-parasite chemotherapy has been given. The Western blot method has been recommended for confirmatory serologic diagnosis of Chagas' disease. The intradermal test by inoculation of extract of *T. cruzi* culture (cruzin), a delayed hypersensitivity reaction is obtained. Finally, a biopsy of the involved lymph node or muscle may reveal the amastigote forms of *T. cruzi*.

Xenodiagnosis

In the indeterminate and chronic phases of the infection, *T. cruzi* is present in such low numbers in the peripheral blood that parasitemia may not be detectable by microscopy even after the concentration methods. In xenodiagnosis, colony-bred uninfected reduviid bugs are fed on a suspect host and dissected about 20-25 days later to detect epimastigotes in the feces, hemolymph, hindgut and salivary glands. Xenodiagnosis is generally favored because, unlike blood culture, it does not require strict aseptic precautions.

Treatment

Nifurtimox and benznidazole may be used for the treatment of American trypanosomiasis. Both these agents suppress the parasitemia and can cure the acute phase of Chagas' disease in 60-80% of cases.