Parasite Biochemistry

Lecture 1

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VAM/Biochem for MBCh.B, BDS & B.PHARM - S1/2017/2018

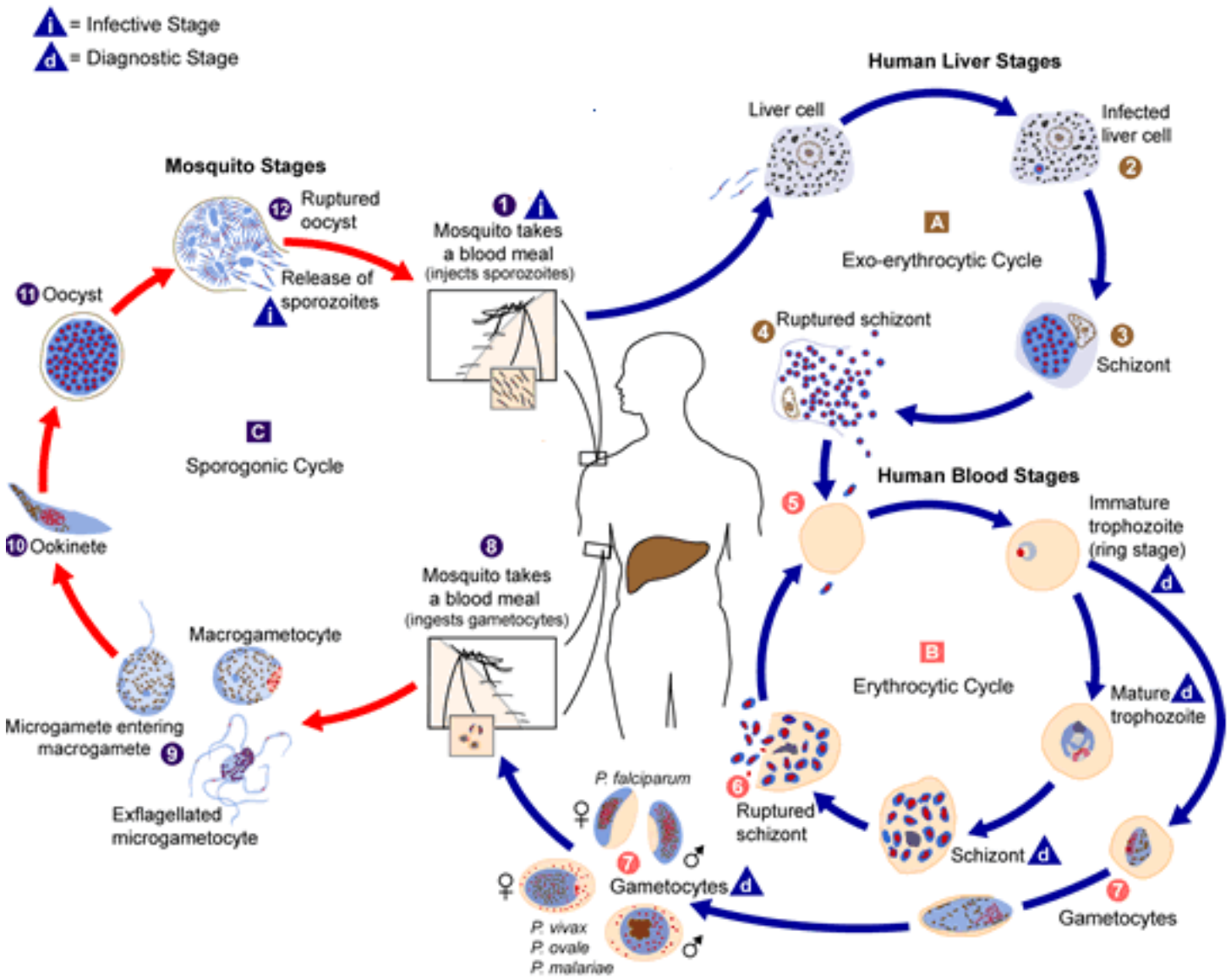
Module Objective

- The objective of this module is to understand biochemistry of eukaryotic parasites. The lecture will feature unique biochemical pathways found in parasites and their potential for exploitation as chemotherapeutic targets

Protozoan parasites

Life cycle of *Plasmodium*

- Human malaria is caused by infection with intracellular parasites of the genus *Plasmodium*, and transmission is achieved through a mosquito vector of the genus *Anopheles*.
- *Plasmodium* species usually have strong specificity for the host species they infect .
- Four species of *Plasmodium* parasites predominantly infect humans: *P. falciparum*, *P. ovale*, *P. vivax* and *P. malariae*. Besides these, *P. knowlesi* which is a natural parasite of macaque monkeys has been found to infect humans commonly in some parts of Southeast Asia.


i = Infective Stage
d = Diagnostic Stage
Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocytic Cycle
3
Schizont
4
Ruptured schizont
Human Blood Stages
5
Immature trophozoite (ring stage)
d
Mature trophozoite
B
Erythrocytic Cycle
6
Ruptured schizont
Schizont
d
7
Gametocytes
P. falciparum
♀
♂
7
Gametocytes
d
P. vivax
P. ovale
P. malariae
♀
♂
8
Mosquito takes a blood meal
(ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete
9
Exflagellated microgametocyte
10
Ookinete
11
Oocyst
12
Ruptured oocyst
Release of sporozoites
i
Mosquito Stages
1
i
Mosquito takes a blood meal
(injects sporozoites)
C
Sporogonic Cycle

- Infected anopheline mosquito injects sporozoites into the human host (1). Sporozoites move through the dermis and then the circulatory system to infect hepatocytes (2) where they undergo multiple rounds of replication generating thousands of merozoites and the infected hepatocytes mature into schizonts (3), which rupture and release merozoites (4).
- After this initial exo-erythrocytic replication cycle [A], the parasites undergo asexual multiplication in the erythrocytes [B].
- The intra-erythrocytic cycle begins with the invasion of merozoites into the erythrocytes (5).
- The ring stage trophozoites mature into schizonts, which rupture releasing merozoites (6).
- Some parasites differentiate into blood stage nonreplicating sexual forms called gametocytes (7).

- Male gametocytes (microgametocytes) and female gametocytes (macrogametocytes) are ingested by an anopheline mosquito during a blood meal (8).
- The parasite undergoes multiplication in the mosquito, the sporogonic cycle [C].
- While in the mosquito's stomach, the microgametes penetrate the macrogametes (9) generating diploid zygotes.
- The zygotes become motile and elongated (ookinetes) (10) which invade the midgut wall of the mosquito where they develop into oocysts (11).
- Mature oocysts rupture and release sporozoites (12), which may reinvade a host following an infectious bite (1).

- Haploid asexual reproduction of the parasite takes place throughout the life cycle except for a brief diploid sexual phase in the female *Anopheles* mosquito
- During mosquito blood feeding an infected mosquito inoculates sporozoites into the human host. Majority of the sporozoites are deposited under the skin and not directly injected into the circulation
- A significant proportion of sporozoites remain in the dermis. Sporozoites use gliding motility to migrate through the dermis tissue and to the liver via the circulatory system, and invade hepatocytes.
- About 30% of the sporozoites that leave the dermis take an alternative route through the lymphatic system. However, most of the sporozoites that invade the lymphatic vessels are trapped in the lymph nodes and despite some of them undergoing partial development similar to the liver stage of the parasite; they are eventually destroyed

- Depending on *Plasmodium* species, over a period of 5-16 days each parasite grows and divides mitotically into tens of thousands of merozoites within schizonts, which rupture and release into the blood circulation and then infect erythrocytes.
- Alternatively, some *P. vivax* and *P. ovale* parasites can remain dormant (hypnozoites) and persist in the liver, and these may cause relapses by invading the bloodstream weeks or years later.
- Blood stage parasites are responsible for the clinical manifestations (for instance, fevers and chills) of the disease.
- Erythrocyte-stage *Plasmodium* parasites undergo repetitive rounds of invasion, growth, and mitotic division every 24 hours (P. knowlesi), 48 hours (*P. falciparum*, *P. vivax*, and *P. ovale*), or 72 hours (*P. malariae*).
- In the erythrocytes the ring stage trophozoites mature into schizonts, which rupture releasing merozoites.

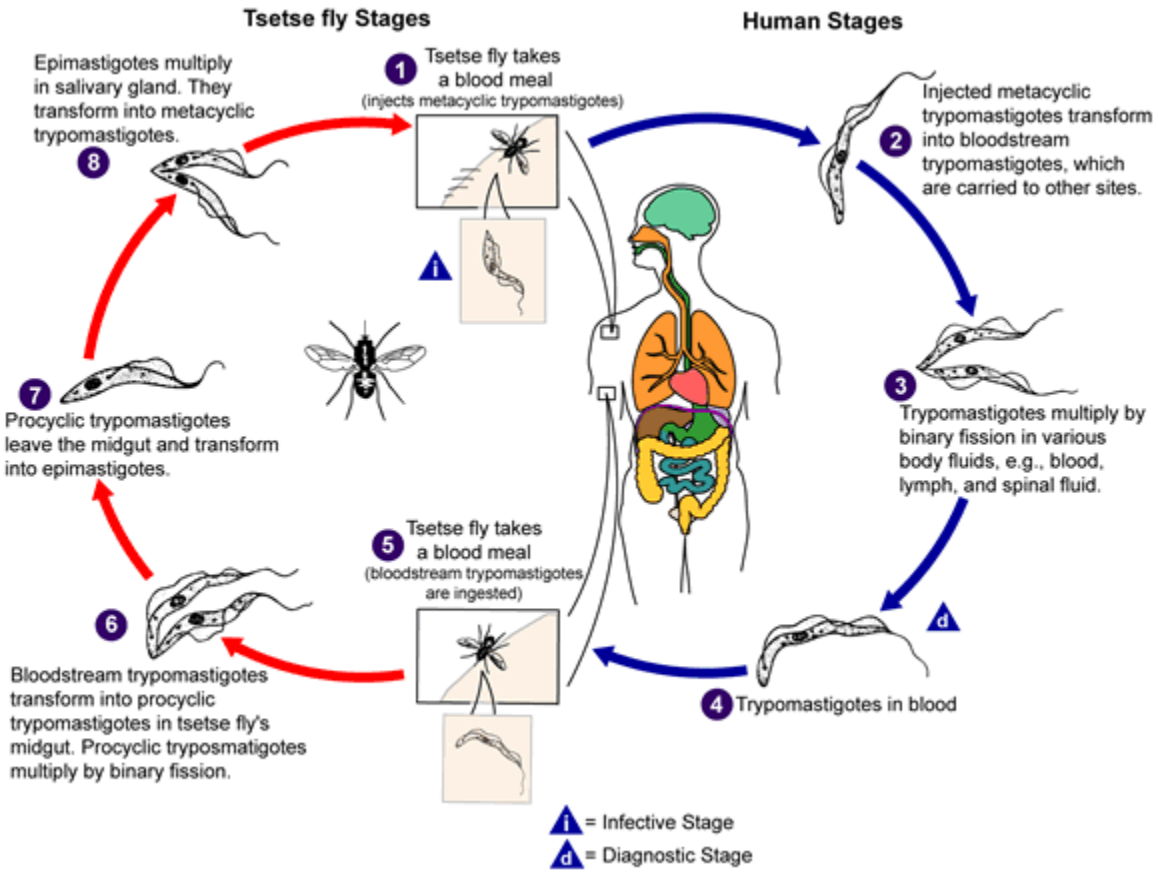
- Some parasites differentiate into sexual erythrocytic stages (gametocytes).
- The commitment to differentiate into male or female occurs in the cycle before the one in which gametocytes differentiate; as the progeny of a single schizont are all either male or female.
- The male gametocytes (microgametocytes) and female gametocytes (macrogametocytes) are ingested by an *Anopheles* mosquito during a blood meal.
- While in the mosquito's stomach, microgametocytes undergo exflagellation to form microgametes which penetrate the macrogametes generating diploid zygotes.

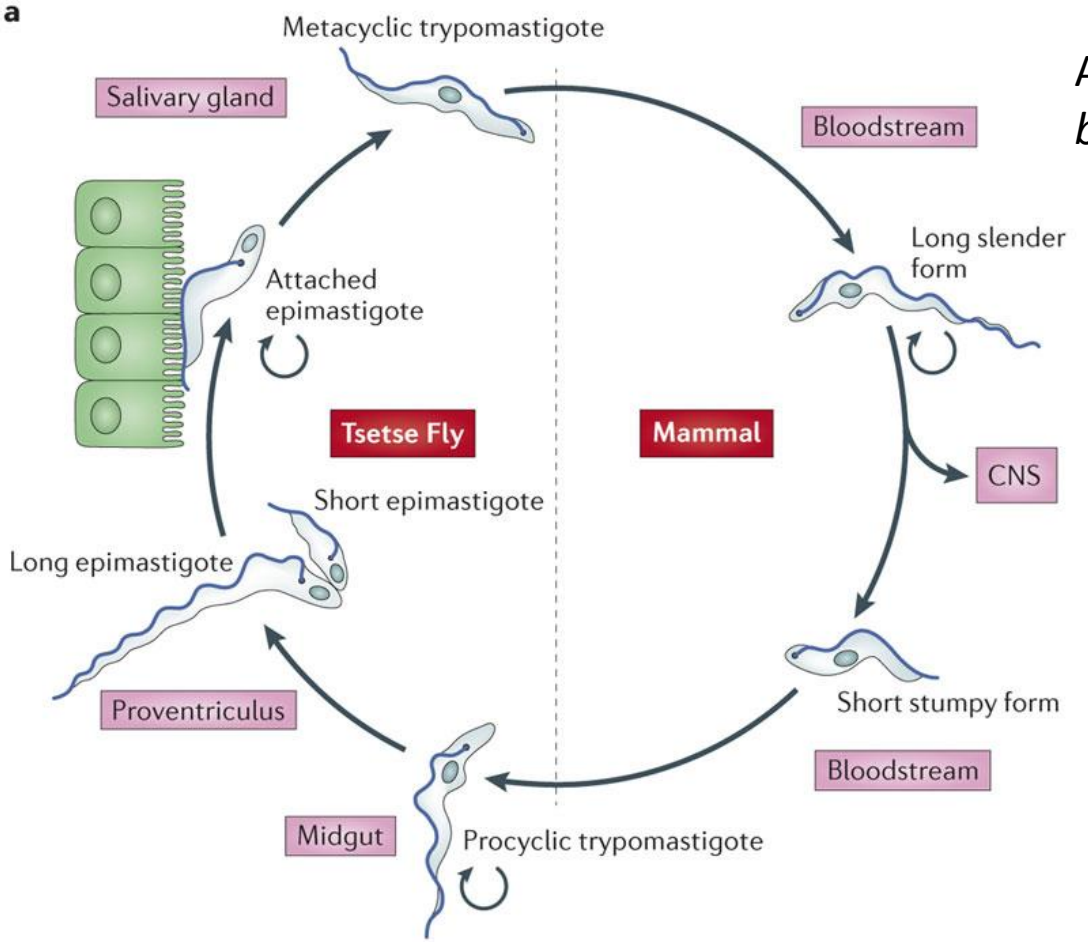
- These zygotes may be a product of fertilization between either genetically different or identical male and female gametes, when there is more than one genotype of parasite in the blood meal.
- The timing of the process of gametogenesis in female gametocytes is critical for fertilization to take place since the male gamete is short-lived.
- Gene disruption of female gametocyte-specific gene that is involved in gametocytes egress from erythrocytes while in the mosquito vector, undermines gametogenesis and may block infection.
- The zygotes formed after fusion of gametes become motile and elongated forming diploid ookinetes which invade the midgut wall of the mosquito where they develop into oocysts.
- Ookinetes divide meiotically to give haploid progeny that then replicate asexually with mitotic divisions within oocysts.

- The oocysts grow, rupture, and release haploid sporozoites that travel to the mosquito salivary glands.
- Inoculation of the sporozoites into a new human host during feeding initiates another infection.

Life cycle of Trypanosomes

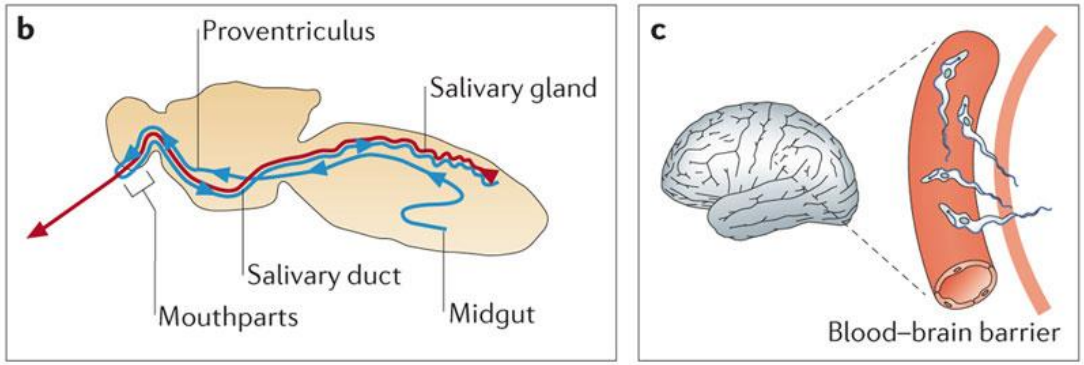
- These are Protozoan hemoflagellates belonging to the complex *Trypanosoma brucei*.
- The African Salivarian trypanosomes are the causative agents of both Human African Trypanosomiasis, or sleeping sickness, and Animal African Trypanosomiasis, more widely known as Nagana
- Two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans: *T. b. gambiense* causes West African sleeping sickness and *T. b. rhodesiense* causes East African sleeping sickness.
- A third member of the complex, *T. b. brucei*, under normal conditions does not infect humans
- The animal infective species *T. congolense* and *T. vivax* are responsible for millions of livestock and wild animal infections across the continent.


Tsetse fly Stages
Human Stages
Epimastigotes multiply in salivary gland. They transform into metacyclic trypomastigotes.
8
1 Tsetse fly takes a blood meal
(injects metacyclic trypomastigotes)
i
2 Injected metacyclic trypomastigotes transform into bloodstream trypomastigotes, which are carried to other sites.
3 Trypomastigotes multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.
d
4 Trypomastigotes in blood
5 Tsetse fly takes a blood meal
(bloodstream trypomastigotes are ingested)
6
Bloodstream trypomastigotes transform into procyclic trypomastigotes in tsetse fly's midgut. Procyclic tryposmatigotes multiply by binary fission.
7
Procyclic trypomastigotes leave the midgut and transform into epimastigotes.
i = Infective Stage
d = Diagnostic Stage



A generalized life cycle of *Trypanosoma brucei*

Can invade extravascular tissues, including the central nervous system (CNS)



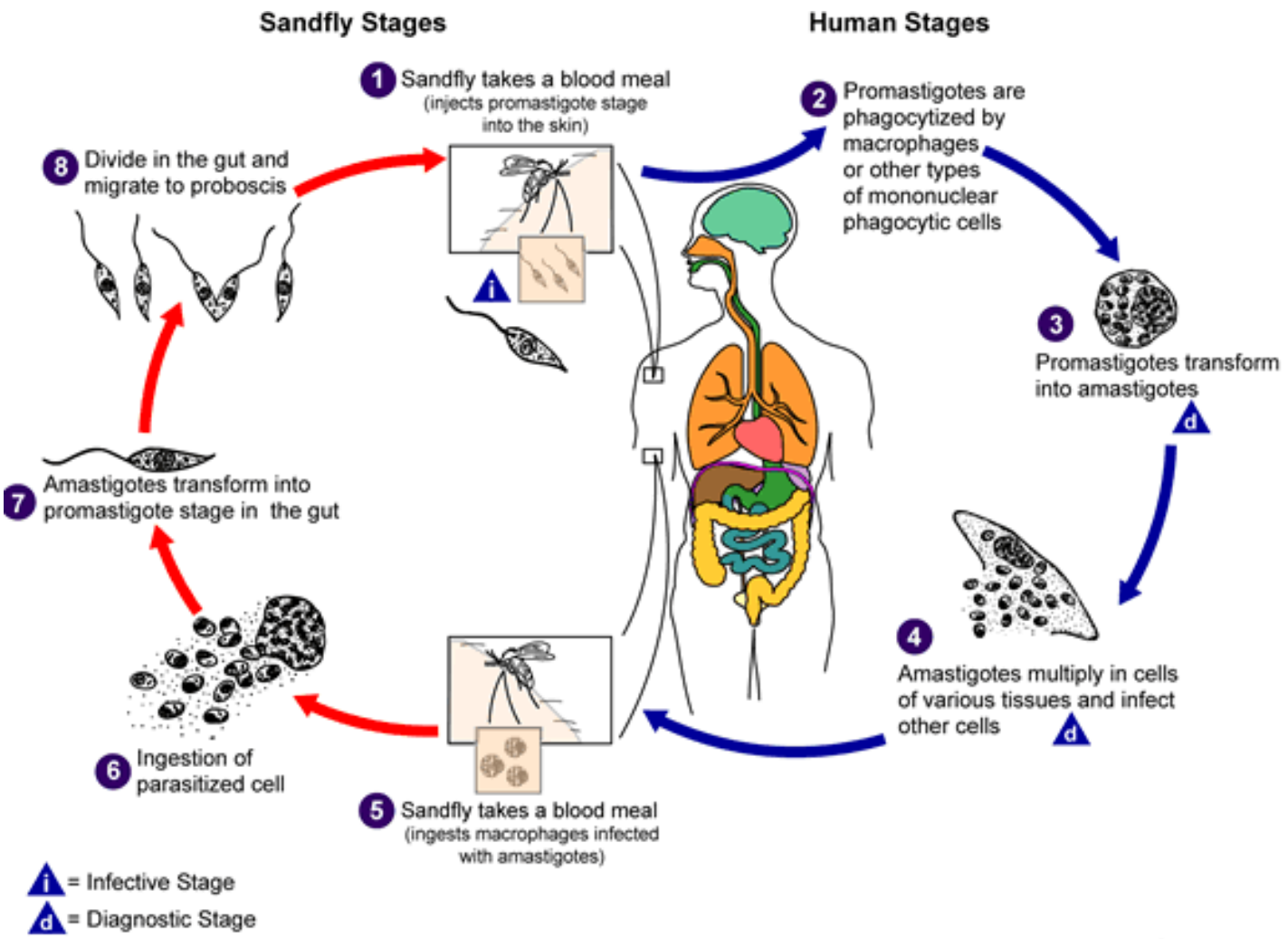
Parasites move out of blood vessels, penetrating the BBB to enter the CNS

- During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue.
- The parasites enter the lymphatic system and pass into the bloodstream .
- Inside the host, they transform into bloodstream trypomastigotes , are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission .
- The entire life cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host.
- In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission , leave the midgut, and transform into epimastigotes .

- The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission .
- The cycle in the fly takes approximately 3 weeks.
- Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals.
- Wild game animals are the main reservoir of *T. b. rhodesiense*.

Life cycle of Leishmania

- Leishmaniasis is a vector-borne disease that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus *Leishmania*.
- Human infection is caused by about 21 of 30 species that infect mammals.
- These include the *L. donovani* complex with 2 species (*L. donovani*, *L. infantum*); the *L. mexicana* complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*); *L. tropica*; *L. major* and *L. aethiopica*.
- The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.


Sandfly Stages
Human Stages
1 Sandfly takes a blood meal
(injects promastigote stage into the skin)
2 Promastigotes are phagocytized by macrophages or other types of mononuclear phagocytic cells
3 Promastigotes transform into amastigotes
4 Amastigotes multiply in cells of various tissues and infect other cells
5 Sandfly takes a blood meal
(ingests macrophages infected with amastigotes)
6 Ingestion of parasitized cell
7 Amastigotes transform into promastigote stage in the gut
8 Divide in the gut and migrate to proboscis
i = Infective Stage
d = Diagnostic Stage

- Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies.
- The sandflies inject the infective stage (i.e. promastigotes) from their proboscis during blood meals .
- Promastigotes that reach the puncture wound are phagocytized by macrophages and other types of mononuclear phagocytic cells.
- Progmastigotes transform in these cells into the tissue stage of the parasite (i.e. amastigotes) , which multiply by simple division and proceed to infect other mononuclear phagocytic cells .
- Parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous (causes skin sores) or visceral (affects internal organs: spleen, liver and BM) leishmaniasis results.
- Visceral leishmaniasis is also called **kala-azar**

- Sandflies become infected by ingesting infected cells during blood meals .

- In sandflies, amastigotes transform into promastigotes, develop in the gut (in the hindgut for leishmanial organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus), and migrate to the proboscis .

Comparative carbohydrate metabolism in Trypanosomes

- The blood stream trypomastigotes forms of *T. brucei* possess no energy stores, are unable to oxidize amino acids or fatty acids and are thus entirely dependent on an exogenous supply of carbohydrate for their energy production.
- In a pleomorphic *T. rhodesiense* infection, 2 morphological forms of the parasite exist: Long slender (LS) and short stumpy (SS).
- The incomplete oxidation of glucose is most marked in LS forms of an infection.
- These organisms catabolize glucose aerobically to pyruvate, with traces of CO_2 and glycerol.
- Pyruvate is not catabolized further as the LS form lacks the enzymes **pyruvate dehydrogenase** and **Lactate dehydrogenase** (LDH)
- However, in the SS forms of *T. rhodesiense*, active enzyme systems for the oxidative decarboxylation of pyruvate have developed.

- These organisms produce a much more varied series of end products from glucose catabolism including CO_2, acetate and succinate.
- In *T. brucei* molecular oxygen serves as the terminal electron acceptor, resulting in the formation of H_2O.
- Glycerol-3-phosphate shuttle transports the reducing equivalents from NADH in the cytosol into the mitochondrion, where via a glycerophosphate oxidase system, it passes to O_2. This enzyme appears to be unique to salivarian trypanosomes.
- Under anaerobic conditions, LS blood stream forms continue to utilise glucose, as under aerobic conditions, but because the glycerophosphate oxidase is inoperative, the glucose is metabolized into equimolar amounts of pyruvate and glycerol.
- The procyclic (vector) forms undergo a metabolic switch, with the development of a fully developed mitochondrion with cristae and most of the Kreb's cycle enzymes although citrate synthase appears to be missing.
- In cultured procyclic forms, glucose is consumed at a slower rate than in the blood stream forms and pyruvate is oxidized further, the main end product being CO_2 , acetate and citrate.

- The enzyme NADH-fumarate reductase (FR) which can reverse the Kreb's cycle by reducing fumarate to succinate is present, a situation that also occurs in parasitic helminths.
- Succinate is also an end product of proline catabolism in the midgut stages of *T. brucei* which closely resemble procyclic culture forms.
- Utilisation of proline correlates well with environment provided by the tsetsefly as the haemolymph contains excessively high levels of proline although deficient in carbohydrates.
- In tsetseflies, therefore trypanosomes depend largely on proline for their energy, by developing NADH-fumarate reductase, they are able to produce succinate which may be used as the main respiratory substrate or as an electron sink to be excreted outside the cell under anaerobic conditions.

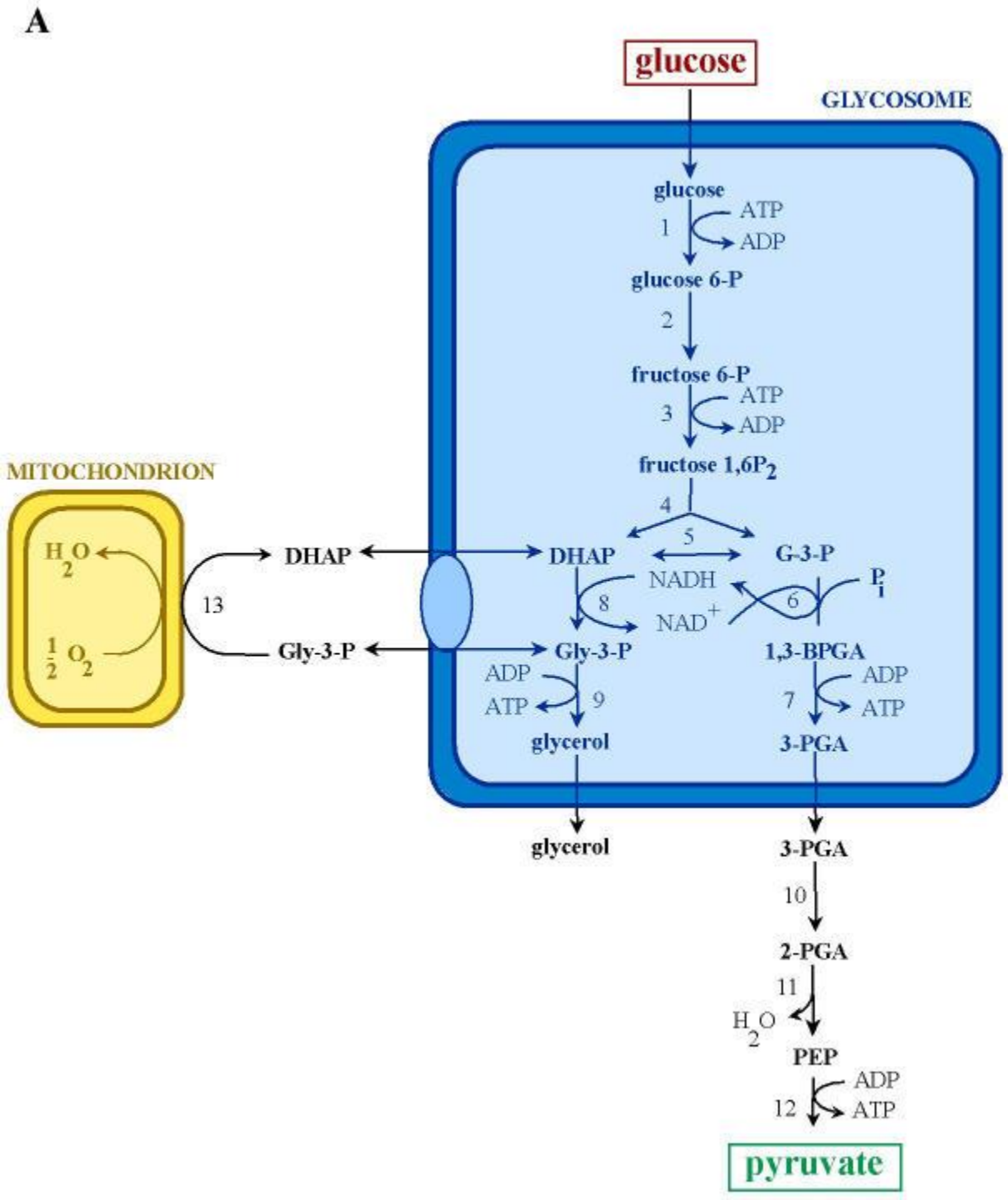


Figure A
The energy metabolism of bloodstream-form of *T. brucei*

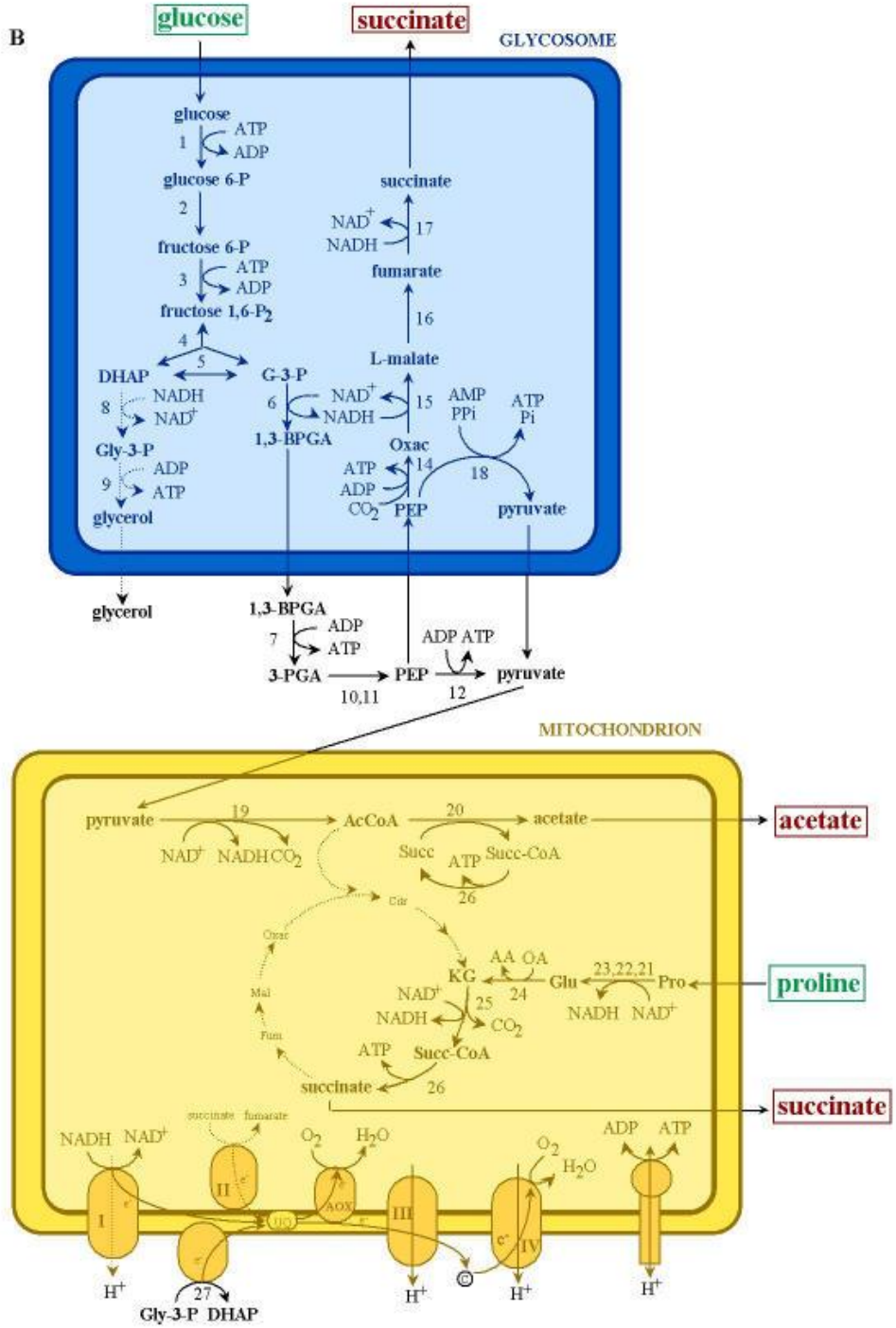


Figure B
The energy metabolism of Procyclic (vector) form of *T. brucei*

Abbreviations:
AA, amino acid; AcCoA, acetyl-CoA; 1,3-BPGA, 1,3-bisphosphoglycerate;
c, cytochrome c; Citr, citrate; DHAP, dihydroxyacetone phosphate;
Fum, fumarate; G-3-P, glyceraldehyde 3-phosphate;
Glu, glutamate; Gly-3-P, glycerol 3-phosphate; KG, α-ketoglutarate; Mal, malate; OA, 2-oxoacid; Oxac, oxaloacetate; PEP,phosphoenolpyruvate; 3-PGA, 3-phosphoglycerate; Succ, succinate; Succ-CoA, succinyl-CoA; UQ, ubiquinone.

Enzymes

1, hexokinase;

2, glucose-6-phosphate isomerase;

3, phosphofructokinase;

4, aldolase;

5, triosephosphate isomerase;

6, glyceraldehyde-3-phosphate dehydrogenase;

7, phosphoglycerate kinase;

8, glycerol-3-phosphate dehydrogenase;

9, glycerol kinase;

10, phosphoglycerate mutase;

11, enolase;

12, pyruvate kinase;

13, glycerol-3-phosphate oxidase;

14, phosphoenolpyruvate carboxykinase;

15, L-malate dehydrogenase

16, fumarase;

17, fumarate reductase;

18, pyruvate phosphate dikinase;

19, pyruvate dehydrogenase complex;

20, acetate:succinate CoA transferase;

21, proline oxidase;

22, Δ'-pyrroline-5-carboxylate reductase;

23, glutamate semialdehyde dehydrogenase;

24, glutamate dehydrogenase;

25, α-ketoglutarate dehydrogenase;

26, succinyl CoA synthetase;

27, FAD-dependent glycerol-3-phosphate dehydrogenase.

- Substrates and secreted end-products are indicated in green and red, respectively, and boxed.
- Enzymes involved in reactions represented by dashed lines are present, but experiments indicated that no significant fluxes occurred through these steps
- A complex II is depicted because succinate dehydrogenase activity and succinate-dependent respiration have been demonstrated in mitochondria of *T. brucei* procyclics and *T. cruzi* epimastigotes. However the role of succinate respiration in the overall metabolism of these cells remains to be clarified.
- No evidence has been reported that electron transfer through complex I of the respiratory chain of trypanosomatids is coupled to proton expulsion. The mitochondrion contains two membranes; the inner membrane containing the respiratory chain and H^+-ATPase has been drawn in the figure above.